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BURLINGTON, VT.

BULLETIN NO. 167

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Micro-organisms of Maple Sap

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BULLETIN 167: MICRO-ORGANISMS OF MAPLE SAP.

Part I. Micro-organisms Occurring in Maple Sap and their Influence on the Color, Flavor and Chemical Composition of Sirup.

By H. A. Edson

Part II. Discussion of Physical and Chemical Data Secured on Maple Sirups Obtained from Saps Inoculated with Micro-organisms.

By C. H. Jones

Part III. Technical Description of Certain Bacteria Occurring in Maple Sap.

A. Description of Bacillus aceris (n. sp.).

B. Brief Description of the Pink Cocci of Maple Sap.

C. The Green Fluorescent Bacteria Occurring in Maple Sap.

By H. A. Edson and C. W. Carpenter

The completion of the studies reported in Part I and in Part III (A) of this bulletin was made possible through the courtesy of the Chief of the Bureau of Plant Industry of the United States Department of Agriculture. The author of those parts, the former station bacteriologist, when he withdrew from station employ to enter that of the Department, had not pursued the investigation far enough to warrant final publication. The Chief of the Bureau generously allowed him to continue his work after he had become connected with the Department during the succeeding sugar season, at intervals during the year, and for several months early in 1912 while preparing the matter for the press. Had it not been for this cordial cooperation, the matter could hardly have been brought to a successful issue; and the Station gratefully acknowledges its obligation for the courtesy.

J. L. Hills, Director.

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PART I

MICRO-ORGANISMS OCCURRING IN MAPLE SAP AND THEIR INFLUENCE ON THE COLOR, FLAVOR AND CHEMICAL COMPOSITION OF SIRUP

By H. A. Edson

Introduction

A preliminary contribution upon the work presented in this paper appeared in bulletin 151 (1910). Only the more important facts reported at that time are restated in the present paper, but quotations and repetitions stripped of details are introduced when essential to a clear and complete presentation. The problem is most satisfactorily introduced, however, by a somewhat lengthy quotation from the former paper, in which, for the information of any who may not be familiar with them, are included brief descriptions of the main facts of sap flow and of sugar making as practiced in Vermont.

"Late in March, in this section, evidences of coming spring appear. The nights are still cold and frosty but the days are genial and the temperature rises a few degrees above the freezing point. If, at this time, the trunk or limbs of certain species of the genus *Acer* are fresh wounded a sweet sap exudes. The Indians were familiar with this phenomenon before white men came; and had learned to collect, to concentrate and to make sugar from this sap. The early settlers[1] learned from them the essential steps which, in modified form, constitute the procedure followed in the maple sugar industry today."

"According to modern practice the tree is tapped by boring a half-inch hole 2 inches deep about 4 feet from the ground. A round, hollow spout or "spile" of wood or metal, upon which is suspended a bucket to catch the dripping sap, is driven into the hole. The sap flow is not continuous but is divided into short intermittent periods, technically termed 'runs.' It occurs only during the three or four weeks which immediately precede the unfolding of the leaf buds. Both its periodicity and

[1] Garden and Forest 4, p. 171.

its duration depend upon weather conditions. The sap is more likely to flow in the daytime than at night; and the more important runs are confined to what are spoken of as 'good sap days.' These occur only after the air temperature has remained below freezing for some time. If, following such a cold spell, the temperature rises materially above 32° F. a good run is likely to ensue. Excessive warmth and high winds check the flow. Freezing nights followed by moderately warm, cloudy days, and the absence of excessive sunshine and heavy winds, are the meteorological conditions which characterize the best sugar weather. So long as the air temperature remains essentially constant, whether warm or cold, little or no sap is obtained."

"The buckets in which the sap is caught are made of wood, tin, or galvanized iron; and, in the better works, are covered to keep out rain, snow and other foreign material. The sap is collected after each day's run and taken to the boiling house, technically known as the sugar house, where it is concentrated into sirup in large shallow pans over a roaring wood fire as rapidly as the capacity of the equipment will permit."

"Maple sap is a sweet liquid containing a varying amount, averaging from 2 to 3%, of sucrose, and, usually, traces of invert sugar. In addition to these carbohydrates it contains small amounts of proteids, of mineral matter, mainly lime, and potash, and of acids, mainly malic acid. The sap of the earlier flows is water clear and transparent, and possesses a clean, sweet flavor. With the advance of the season, however, it undergoes a marked change. As the days grow warmer and night freezes are less severe and less frequent, the sap gradually becomes cloudy and discolored and unpleasant flavors develop. Such sap, while usually containing only the normal amount of acid, is popularly termed 'sour.' It rapidly deteriorates when stored even for a few hours. Several types of sour sap are recognized by sugar makers, to which the descriptive terms 'milky,' 'stringy,' 'red,' and, particularly, 'green' are commonly applied. Green sap is almost always secured just before the close of the season, when

the leaf buds are ready to open. It is popularly believed that the swelling of the buds, associated with the renewal of vegetative activity in the tissues of the tree, is accompanied by a change in the composition of the sap within the trunk; and that the alteration in color and flavor are manifestations of this change. The term 'buddy' is universally used to describe this sort of sap." (See Plate V).

"The sirup made from late runs is much inferior to that derived from the earlier flows. 'Last run' goods are often very dark in color and usually lack the delicate flavor possessed by the best sirups. Moreover, the quality of sirup varies markedly from year to year and these variations are seldom local in distribution. Such widespread fluctuations in quality are not accidental but of necessity must be associated with some fundamental cause or causes. It is conceivable that they may be related to weather conditions, either during the preceding summer or during the progress of the sugar season. It is known, moreover, that inferior products result from carelessness and lack of cleanliness in collecting and handling maple sap. Such procedures must occasion a great increase in the bacterial content of the sap, just as in dairying they entail serious bacterial contamination in milk. The proteid, carbohydrate and mineral contents of maple sap are sufficient to make it a fairly good medium for the development of bacterial life, provided suitable temperature relations are maintained; and the vital activities of large numbers of micro-organisms would presumably affect the flavor and quality of the sirup produced under such conditions."

"Reflection upon these facts strongly suggests the possibility that micro-organisms may be associated with the inferiority of the maple output in all the cases cited, or that, indeed, they may be the direct cause of the troubles. Inevitably they must be present in the sap and it is to be expected that they would be more abundant toward the close of the sugar season than earlier, because the warmer weather would favor their increasingly rapid development and multiplication. It is conceiv-

able that the 'off' seasons may prove to be those wherein conditions foster this microscopic life to an unusual degree. More favorable temperature relations, or longer periods of incubation, or both, in 'off' years and in the latter part of the season, might reasonably be expected to promote the multiplication of organisms in the tap-hole, spout and bucket, thus producing heavy initial inoculation of the sap. Uncleanly methods would certainly result in this condition, and in any case, storing, even for a short length of time, would serve to increase the troubles due to the vital activities of microscopic organisms, particularly if the temperature of the storage house was considerably above the freezing point, as is apt to be the case."

The experimental work upon which a report is submitted in these pages was undertaken in an effort to determine as far as might be possible the answers to the following questions: What is the cause of the deterioration of maple sap? Is it due to changes in composition occurring within the tissues of the tree as a result of the resumption of protoplasmic activities and vegetative vigor? Is it due to the action of micro-organisms in the sap after it leaves the vascular bundles of the trunk? Or is it to be attributed to a combination of these causes?

THE RELATION OF MICRO-ORGANISMS TO SPOILED MAPLE SAP

The early studies, previously reported, have shown that maple sap while in the vascular bundles of the tree is sterile, and that when drawn without contamination and stored under aseptic conditions it keeps perfectly for years, even with free access of air. Hence the changes in sap known as souring are not to be attributed to the action of enzyms present as natural ingredients or to other forms of auto-decomposition. Careful studies of the microscopic flora of spoiled sap have shown that micro-organisms are constantly associated with the process of souring.

In certain types of spoiled sap specific groups of organisms are likely to predominate, while in other types several of the

common forms may be found growing together in association. In addition to various common saprophytic bacteria, wild yeasts of several types, and green molds of the genera Penicillium and Eurotium are frequently met with, particularly towards the close of the season when the temperature becomes more favorable for their development. Green sap, for example, is associated with the presence of a group of bacteria characterized by green fluorescence and does not appear to be caused by the swelling of the buds to which it is so frequently related in time. In one type of stringy sap occasionally found, the only significant organism was one described in this paper as *Bacillus aceris* (new species), while in another type of stringy sap common at the close of the season, several species of bacteria, various wild yeasts, and molds of different genera occurred in association. Gray and red yeasts are commonly associated with bacteria in so-called red sap. Spore-bearing bacteria of the hay bacillus type were frequent in certain samples of milky sap examined, while the condition of other samples seemed attributable to the collective action of many different organisms.

The conclusion stated in the preliminary publication, that there is a causal relation between micro-organisms and sour sap and the resulting poor sirup, has been confirmed by all the examinations made during the progress of the work, and by the inoculation experiments to be reported later. A sequence of species was noted during the season of 1909, and discussed on page 494 of bulletin 151 where the following sentences occur: "The predominating organisms found during the early days of the sugar season belong to the yeast-like group, while bacteria were relatively few in numbers. As the season advanced these conditions were reversed." Subsequent studies have not confirmed this observation. For the past two years relatively few organisms of any kind have appeared on the plates early in the season, but as a rule bacteria have predominated, and in general yeast-like organisms have appeared in important numbers only after the season was well advanced.

THE NUMBER OF MICRO-ORGANISMS IN MAPLE SAP

During the first two seasons covered by these studies (1907-and 1908) no attempt was made to determine the bacterial count in maple sap of different types. Attention was rather directed to determining the predominating forms of micro-organisms present, and to securing cultures to be employed as a basis for the morphological and biochemical studies and for inoculation work, which have constituted the major portion of the problem. In connection with the field studies of the past three years, however, some attention has been given to collecting data on the number as well as the character of the micro-organisms present. The time devoted to this feature of the work was limited and the data secured is consequently more or less fragmentary, but it appears of sufficient importance to warrant presentation. The material examined was secured from several sugar places and at various intervals during the season.

For the purpose of enumeration the ordinary poured plate method with dilutions was employed. Two types of media were used. The first was ordinary nutrient agar; the other, a synthetic agar, less well adapted to the cultivation of bacteria than is the ordinary agar but suited to the development of yeasts and molds. It had the following composition:

Water	1000	parts
Dextrose	100.	"
Peptone	20.	"
Ammonium nitrate	2.5	"
Magnesium sulphate	5.	"
Potassium nitrate	2.5	"
Potassium phosphate	2.5	"
Calcium chlorid	0.1	"
Agar	15.0	"

It should be understood that the figures given in the tables immediately succeeding are only approximate and that in most cases they are probably below the maximum because of the fact that the dilutions employed were usually insufficient to prevent serious crowding on the plates. The blank spaces in the column headed "synthetic agar" may be taken as an indication that the sap in question was plated only on nutrient agar.

NUMBER OF ORGANISMS IN SOUR MAPLE SAP

The results obtained with various samples of sour sap plated at one or another time during the progress of the work are given below in tabular form. Some of the material plated was just beginning to show signs of clouding while in other cases the process was well advanced. In general the degree of decomposition and the number of organisms found bore a direct relation to each other.

As may be seen from the table the count obtained on nutrient agar varied from 320,000 to 141,420,000 per cc. Allowing an average size of 1 micron by 3 microns the organisms represent approximately one-third the volume of the most heavily infected sap.

TABLE 1. ORGANISMS PER CC. IN SOUR MAPLE SAP

No.[1]	Nutrient agar	Synthetic agar	No.	Nutrient agar	Synthetic agar
1	3,084,000	200,000	27	14,000,000	9,546,000
2	6,100,000	2,000	28	5,667,000	51,000
3	21,300,000	.....	29	4,225,000	636,000
4	6,300,000	13,000	30	19,500,000	.....
5	1,272,000	.....	31	23,000,000	15,000
6	2,785,000	.....	32	9,750,000	37,000
7	320,000	715,000	33	325,000	.....
8	2,000,000	30,000	34	4,875,000	.....
9	843,000	200,000	35	10,400,000	.....
10	975,000	175,000	36	43,875,000	.....
11	8,276,000	20,000	37	11,275,000	.....
12	1,950,000	1,300	38	56,250,000	.....
13	6,500,000	500,000	39	2,250,000	.....
14	19,092,000	.....	40	1,625,000	.....
15	1,357,000	3,000	41	12,350,000	10,500
16	1,909,000	254,500	42	3,262,500	407,000
17	1,751,000	.....	43	23,400,000	60,000
18	10,000,000	.....	44	29,250,000	65,000
19	88,200,000	.....	45	11,700,000	646,300
20	1,950,000	.....	46	9,480,000	.....
21	14,500,000	2,450,000	47	14,670,000	.....
22	8,850,000	10,000	48	6,500,000	.....
23	7,300,000	500	49	73,125,000	87,750,000
24	2,150,000	30,000	50	65,000,000	100,000
25	5,540,000	17,500	51	13,000,000	3,640,000
26	7,500,000	.....	52	141,420,000	.....

[1] This series, 1-52, is entirely distinct from the similarly numbered series later discussed on pages 351 to 365.

NUMBER OF ORGANISMS IN SWEET MAPLE SAP

In contrast to the figures in the preceding table the results obtained from plating several samples of normal sap are of interest. In some cases the sap to be analysed was gathered with unusual care in order to secure as light an infection as might be without special apparatus, while in other cases it was taken from the commercial supply and is representative of the ordinary sap of the orchard in which the work was done. These two classes of material are grouped in separate columns. The higher figures in the column headed "commercial sap" were obtained late in the season from material which was developing an unpleasant flavor and which clouded promptly when placed in storage for a few hours. In one of these cases (11) the count obtained is higher than that from many of the sour saps reported above, and in the other four (4, 5, 10, 12) it is very high.

TABLE 2. ORGANISMS PER CC. IN SWEET MAPLE SAP

Carefully collected sap			Commercial sap		
No.	Nutrient agar	Synthetic agar	No.	Nutrient agar	Synthetic agar
1	120	140	1	1,300	16
2	100	45	2	3,700	1,000
3	500	160	3	3,800	700
4	5	0	4	32,500	10,000
5	30	8	5	162,000	325,000
6	66	17	6	900	610
7	59	7	7	1,000	500
			8	140	140
			9	220	220
			10	66,600	44,000
			11	1,000,000	1,100
			12	200,000	.....
			13	2,600	2,700
			14	9,290	784

The influence of cleanliness upon the number of organisms developing in maple sap may be seen in the results obtained in the following experiments.

Several trees were tapped with care, precautions being taken to prevent the introduction of inert matter carrying micro-

organisms into the tap-hole. Sterile metal spouts and buckets were used and the covers employed were fastened to prevent blowing in the wind. The sap was gathered daily, and after each collection the buckets were thoroughly scalded in order to maintain practical sterility. The plates poured from sap obtained on March 26 and April 6 appear in table 3.

TABLE 3. ORGANISMS PER CC. IN SAP OBTAINED IN STERILIZED CONTAINERS

Date	Tree	Nutrient agar	Synthetic agar	Date	Tree	Nutrient agar	Synthetic agar
3/26/11	A	3		4/6/11	A	6	10
"	B	6		"	B	4	2
"	C	250		"	C	3	6
"	D	0		"	D	4	2
"	E	30		"	E	0	5
"	F	2		"	F	2	32

BACTERIAL CONTENT OF SAP OBTAINED UNDER SEPTIC CONDITIONS AS COMPARED WITH THAT OBTAINED UNDER ASEPTIC CONDITIONS

Trees which were running sour sap were retapped about 4 inches to one side of the old tap-hole. Sterile spouts and buckets were hung at the fresh wounds, while the sour spouts and buckets were allowed to remain undisturbed except that the sap was emptied from the old bucket at the time the clean one was hung at the fresh tap-hole. The sap was allowed to run from each spout for a few hours, after which samples were collected and plated. The results are tabulated below in table 4.

TABLE 4. ORGANISMS PER CC. IN SAP FROM FRESH AND FROM SOUR TAPHOLES

	Fresh tap		*Sour tap*	
Tree	Nutrient agar	Synthetic agar	Nutrient agar	Synthetic agar
G	5	0	73,125,000	87,750,000
H	8		4,875,000	
I	70		9,750,000	
J	12		3,250,000	
K	10		1,300,000	

INFLUENCE OF TAP-HOLE INFECTION UPON THE FLORA OF SAP

For the purpose of this series of experiments 6 trees were tapped with unusual precaution to avoid infection in the hole at the time of tapping. The spouts used were of galvanized iron and were thoroughly sterilized before using, but remained undisturbed and without protection, other than the bucket cover, during the season. Bright tin buckets provided with covers were used. They were thoroughly washed and scalded at frequent intervals, usually daily, in order to prevent the accumulation of organisms, which otherwise occurs in the buckets as the season advances. There was, of course, the usual opportunity for increasingly heavy contamination of the flowing sap by organisms developing in the tap-hole and spout. The interval between scalding the bucket and plating the sample varied somewhat with the temperature but was usually about five hours. The progress of infection is shown in table 5. It is interesting to note that most of the colonies developing in the sap from tree number 3 were of a single species. It seems probable that this organism was carried into the tap-hole at the time of tapping and became established there to the practical exclusion of other species.

TABLE 5. ORGANISMS FROM TAP-HOLE AND SPOUT PER CC. OF MAPLE SAP

Date	Tree	Nutrient agar	Synthetic agar	Date	Tree	Nutrient agar	Synthetic agar
3 20 10	1	1	4	3/25/10	1	2	2
"	2	4	0	"	2	4	12
"	3	940	680	"	3	12600	12600
"	4	2	2	"	4	2	6
"	5	4	4	"	5	7	3
"	6	50	1	"	6	11	10
3 22 10	1	2	2	4/4/10	1	1300	910
"	2	40	4	"	2	400	0
"	3	1170	1040	"	3	292500	300000
"	4	6	1	"	4	1300	0
"	5	10	3	"	5	100	2
"	6	4	2	"	6	1000	16

The figures given above indicate that in most cases the infection in tap-hole and spout is slight during the early part of

the season but that it becomes more serious as the spring advances. In the case of tree number 3, however, there is a gradual, constant increase in the infection from the very first.

Influence of the container on the bacterial flora

The influence of the container upon the bacterial flora of sap is strikingly illustrated by the following experiment. On March 22, 1910, the sap which had flowed from the 6 trees mentioned in the previous table during parts of March 21 and 22, was mixed together and plated. The number of organisms per cc. upon nutrient agar was 120, on synthetic agar, 140. Most of these were of the type characteristic of tree 3 as noted above. On the morning of March 23, old but sound wooden buckets previously used in the orchard were thoroughly washed and hung at the 6 trees. The sap was collected, mixed and plated on the afternoon of the next day. The counts on nutrient agar averaged 6,500,000, and on synthetic agar, 500,000. The temperature and general weather conditions during the four days covered by this experiment were essentially similar. That this increase of organisms was due to the buckets employed may be seen by reference to the bacterial count given in table 5 for March 25, when clean tin buckets were again employed. While the evidence goes to show that the initial infection from the taphole of tree 3 was greater on March 23 and 24 than on March 21 and 22, there is no indication that this was the case with the other trees. It should also be noted that the plates poured on March 24 contained no large proportion of colonies characteristic of the organism of tree 3, and that the quality of sirup obtained (number 67 page 372) was not characteristic of the organism in question; hence it would seem that the container must be regarded as the chief source of the increase. See Plates VI and VII.

General plan of field experiments

Having established the fact that large numbers of bacteria and other micro-organisms are constantly associated with spoiled

sap, it remained to determine the influence of these agents upon the quality of sirup. Several hundred pure cultures of the predominant organisms occurring in various types of spoiled sap were isolated and studied more or less critically. After their character had been roughly determined, certain ones were selected for the inoculation experiments to be described presently.

The Orange county sugar orchard in which the field experiments were carried out is situated upon a western slope but is exposed to north winds. The soil of the greater part of the place is wet, and the orchard has had the reputation of producing a grade of sirup of only medium standard both in flavor and color. See Plate I.

The general plan followed during the first season was to secure sap as free from bacteria as was feasible without sterilization, and to introduce a sufficiently heavy inoculation of the specific organism to produce an overgrowth of the introduced species. In some of the later series pasteurization was employed before inoculation, and during the last season fractional sterilization was attempted. Following inoculation, after a period of incubation, the saps were concentrated to sirups under uniform conditions. The sirups were placed in glass jars, sterilized, sealed and shipped to the station bacteriological laboratory at Burlington, where they were stored in the dark for later scoring as to color and flavor, and subjected to a complete chemical analysis. As suggested above, the details of handling the sap before and during incubation varied somewhat from time to time. The details of these variations will be explained with the discussion of the individual samples.

In addition to the inoculation experiments referred to, which were usually confined to the early runs of the season, an attempt was made to procure late run sap relatively free from infection, in order to compare it with material drawn at the same time from the same trees under ordinary conditions. For this purpose trees which had begun to run sour were selected and, without disturbing the original tap-hole, spile or bucket, another spout

was introduced into the tree about 4 inches to one side of the original wound. Great care was observed to secure for this tap-hole as entire freedom from infection as was possible without special apparatus, and sterile spouts and buckets were employed. At the time of retapping, the sap in the sour bucket was emptied but the receptacle was not washed. In this way two samples of sap were obtained from the same tree at the same time, one of which was relatively free from micro-organisms, while the other was heavily infected. These two saps were concentrated to sirup under practically identical conditions and submitted to the same subsequent treatment as were the other sirups. These experiments were intended to show whether the changes which have been demonstrated to occur in sap near the close of the season were due entirely to the action of foreign organisms, or whether they should be ascribed in part or in entirety to physiological changes within the tree.

Method of sirup scoring

At the close of each season the sirups which had been made during the preceding weeks were scored for flavor by a commercial expert who was entirely unfamiliar with their history. A series of numbered beakers of uniform appearance were placed on a table and each received a few ounces of the sirup corresponding in number to that of the beaker. The expert tasted the sirups and assigned each to its respective grade. After the entire series had been scored, and a record made of the results, the samples were rearranged in such a way as to insure the loss of their identity, except by the small number on each beaker, and the judge was asked to regrade them. In cases of special importance the samples were repeatedly disarranged and passed back to be regraded. Incredible as it may seem they were invariably placed in the grades originally assigned them and usually in the same relative position within the grades. Not a single instance of contradiction occurred among the entire 128 samples.[1]

[1] The Station is under obligation to Mr. Otto Ludwig of Burlington for his invaluable and expert assistance.

Six grades of flavor were recognized, the last two of which were reserved for material possessing the characteristic flavor known to experienced sugar makers as "buddy." The term "buddy" as employed in this bulletin should be understood to designate that peculiar flavor which occurs only in sirup made from late run sap drawn after the buds have begun to open. Much of the material popularly spoken of as "buddy" does not possess this distinctive character, which is difficult to describe but is instantly recognized by one who has become familiar with it.

Number 1 sirups possess a very high degree of excellency.

Number 2 sirups are also excellent but are slightly inferior to number 1.

Number 3 sirups possess the characteristic maple flavor but, in addition, there is present an unpleasant taste which detracts from their value.

Number 4 sirups lack maple flavor, possess an unpleasant foreign taste to a marked degree, and are to be regarded as of very poor quality.

Number 5 applies to sirups which possess the "buddy" flavor but are otherwise excellent.

Number 6 sirups combine the "buddy" flavor with the other foreign flavors previously mentioned.

Sirups of grades 5 and 6 can be marketed only with difficulty, and cannot be added to higher grade sirups even in small amounts without rendering the entire mixture undesirable for table purposes. Number 4 sirup does not find a ready market for domestic use unless mixed with higher grade goods.

The sirups were also graded carefully according to color. The method employed for color determination is that suggested by Bryan. The colors consist of a series of twenty standards for the preparation of which Bryan gives the following directions.[1]

[1] U. S. Dept. Agr., Bu. Chem., Bul. 134, p. 15 (1911).

"The materials used are (1) pure glycerin and (2) a caramel solution, which is prepared as follows:

Heat 6 grams of pure sugar to 212° C. for one-half hour in a flat bottomed aluminum dish and dissolve the caramel formed in boiling water, evaporate to a small volume, and make up to 200 cc. with glycerin. The oven for caramelizing the sugar (fig. 1) is constructed as follows:

A and A′ are heavy sheets of asbestos board 18 cm. (7 inches) square, A′ being perforated near one edge by a hole for a cork supporting the thermometer d; b is a sheet-iron cylinder 15 cm. (6 inches) in diameter;; c is a tin can 9 cm. (3½ inches) in diameter, which is filled with paraffin to within 1 cm. (½ inch) of the top. This can rests on the pipe-stem triangle e. The bath or oven is supported on a tripod and is heated by two burners. One burner is so adjusted as to keep the bath at 212° C.

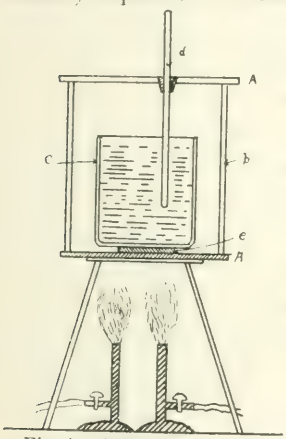

Fig. 1. Apparatus for preparation of standard caramel.

Bring the temperature of the oven up to 212° C., using both burners. Then remove the asbestos cover carrying the thermometer and place 6 grams of sugar in a flat-bottomed aluminum dish 7 cm. (2¾ inches) in diameter and 1.5 cm. (⅝ inch) deep, and put it in the can containing the paraffin. Replace the cover at once and as soon as the temperature reaches 208° C. turn out one burner and keep the bath at 212° C. by carefully adjusting the other one. At the expiration of thirty minutes from the time the sugar was placed in the bath, dissolve in boiling water, and make up as described. The aluminum dish should not be less than 1.5 cm. (⅝ inch) deep, since the sugar melts before caramelizing and runs to one side of the dish, which, if too shallow, will tilt, fill with paraffin, and sink.

With the ingredients thus prepared, the scale of colors is made up by mixing as indicated in the following table:"

TABLE 6. AMOUNTS OF INGREDIENTS TO BE USED IN PREPARING SOLUTIONS FOR THE COLOR SCALE

Color number	Caramel solution, grams	Glycerin, grams
1	0.00	35.00
2	0.25	34.75
3	0.50	34.50
4	0.75	34.25
5	1.00	34.00
6	1.50	33.50
7	2.50	32.50
8	3.50	31.50
9	4.50	30.50
10	5.50	29.50
11	7.00	28.00
12	8.50	26.50
13	11.00	24.00
14	14.00	21.00
15	17.00	18.00
16	20.00	15.00
17	23.50	11.50
18	27.00	8.00
19	31.00	4.00
20	35.00	0.00

The standard colors were placed in screw-capped vials of perfectly clear glass having the same internal diameter. The samples to be examined were placed in similar vials and the colors compared by transmitted light.

For commercial purposes sirups are usually divided into three grades of color. The point of division between the standards fluctuates somewhat according to the locality and season. In a good season and in a locality where light sirups are produced, numbers up to and including 7 are regarded as first quality, while number 11 represents the dividing line between second quality and third. On the other hand in years or in localities characterized by dark sirup, the first eleven grades of color may be accepted as first quality, while number 15 becomes the dividing line between second and third qualities.

In addition to the rating for color and flavor a numerical score card was prepared in which color and flavor were both considered. In scoring, number 1 was ranked as 100, number 2 as 95, and so on, subtracting 5 from the score for each fall of

1 in grade. A number 20 sirup therefore received a value of 5 and anything darker than 20 a value of 0. A table of values is given below.

TABLE 7. VALUES ASSIGNED TO COLOR GRADES

Color	Value	Color	Value
1	100	11	50
2	95	12	45
3	90	13	40
4	85	14	35
5	80	15	30
6	75	16	25
7	70	17	20
8	65	18	15
9	60	19	10
10	55	20	5
		20+	0

In scoring for flavor certain samples were found which clearly were not sufficiently fine to be ranked as number 2 and at the same time were superior to number 3. Two fractional numbers were used to designate these groups. These were 2^1 and 2^2. Similarly one group was introduced between 3 and 4, which is designated as 3^1. In preparing the numerical score card the following table of values was employed:

TABLE 8. VALUES ASSIGNED TO FLAVOR GRADES

Flavor	Value	Flavor	Value	Flavor	Value
1	100	2^2	80	4	50
2	90	3	75	5	20
2^1	85	3^1	65	6	0

The numerical score was obtained by multiplying by 5 the sum of the numerical values assigned for color and for flavor. The highest score theoretically possible is thus seen to be 1,000, but as a matter of fact no maple sirup has the color of clear glycerin so that a sirup of number 1 color is not to be expected, and the highest attainable score becomes 975.

INOCULATION EXPERIMENTS IN 1909

The first inoculations were made in 1909 and were carried out at a farm house some distance from the sugar woods where

most of the field studies were conducted. The sap was collected in clean tin buckets provided with japanned covers. The spouts used were of galvanized iron. Care was exercised to keep the buckets clean and sweet in order to avoid an accumulation of micro-organisms in the sap. The sap was gathered in clean tin cans such as are usually employed for transporting milk, and carried to the farm house in which the temporary laboratory was then established. It was divided into portions of sixteen quarts each and placed in new tin buckets provided with covers. Certain portions were reserved as controls and the remainder were inoculated by adding 70 cc. of a young actively growing culture of the specific organism selected. The controls were treated with similar amounts of sterile culture media. The inoculated samples were placed on a table and incubated for three days. The temperature variation during the respective incubation periods is shown in the accompanying graphs taken from the tracings made by a self-recording thermometer placed near the samples. The actual temperature of the sap was of course much more constant than that of the air. After an incubation period the various portions were made into sirup, each of the several saps being evaporated in a bright sugaring-off pan on a kitchen stove until condensed to a volume of about one quart. It was then transferred to a white, agate-ware basin, and evaporated as rapidly as possible until the proper concentration, as indicated by a thermometer, was reached. The process from cold sap to sirup, required about an hour and a half for its completion, except in the case of one sample when the time was intentionally prolonged. The samples made during the first season are those discussed in bulletin 151 as numbers 1 to 26 inclusive. The numbers used at that time, however, have not been retained in the present publication. Moreover the method of scoring employed in this article is not the one used in the former issue, a fact which explains any apparent lack of uniformity. The numbers used in the present bulletin and in the former one are given below in tabular form:

New number (Bul. 167)	Old number (Bul. 151)	New number (Bul. 167)	Old number (Bul. 151)
1	1	14	18
2	7	15	20
3	8	16	19
4	9	17	21
5	10	18	22
6	2	19	23
7	12	20	5
8	11	21	6
9	13	22	24
10	14	23	26
11	16	24	3
12	15	25	25
13	17	26	4

Since it was deemed important to conduct the inoculation experiments with sap from the first runs only, about 100 trees were tapped for experimental purposes as early as it seemed probable that any sap could be obtained.

DETAILED DISCUSSION OF SAMPLES

SERIES I: SIRUPS 1 TO 6

The sirups of this series were made from the first run of the season which occurred on March 24, 1909. It remained in the woods over night and was brought to the field laboratory the following morning and divided into six portions. Four of these were inoculated and two retained as controls. They were held for three days, the controls being kept at a temperature of from 0° to +10° C. The incubation temperature of the inoculated samples is indicated in the temperature graph (fig. 2).

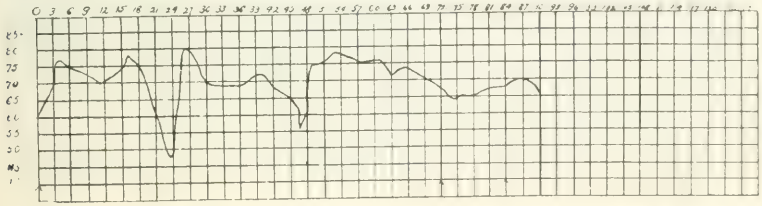

Fig. 2. Graph of incubation temperatures for series 1; saps 1 to 6 inclusive. The arrow heads on next to the bottom line from left to right indicate respectively the time of inoculation, the time of evaporation of the first sample and the time of evaporation of the last sample.

The inoculated saps promptly developed a cloudy appearance and showed all the characteristics of so-called sour sap of the late runs. (See Plate V). The details of the various samples follow:

1. Number 1 was a control used to determine the influence of long boiling upon the quality of the product. The sap was held between three and four days at a temperature only slightly above freezing. At the end of that time it appeared to be as fresh and sweet as when brought from the woods. It was evaporated to sirup over so slow a fire that six hours were required to concentrate the 16 quarts of sap which constituted the sample. Calculated to a dry matter basis, the sirup contained 96.84% sucrose and 1.07% invert sugar. The color was 7, flavor 1, and grade 850; depreciation from control, (No. 6) color 1, flavor 0, and score 25.

2. Number 2 was inoculated with fluorescent organism XXXIII[1]. It developed a cloudy appearance with the greenish cast characteristic of the so-called green sap. The sirup contained 96.79% sucrose and 1.55% invert sugar. The color was 14, flavor 4, and score 425; depreciation from control, color 8, flavor 3, and score 450.

3. Number 3 was inoculated with fluorescent organism XXXVI. It developed the characteristic green type of souring. The sirup contained 96.73% sucrose and 2.09% invert sugar. The color was 11, flavor 2, and score 700; depreciation from control, color 5, flavor 1, and score 175.

4. Number 4 was inoculated with fluorescent organism L, and developed a green type of souring. The sirup contained 96.54% sucrose and 1.56% invert sugar. The color was 9, flavor 2, and score 750; depreciation from control, color 3, flavor 1, and score 125.

5. Number 5 was inoculated with fluorescent organism LIII. It developed typical green souring. The sirup contained

[1] See Part III c, pages 521 to 599 for a description of the members of the fluorescent group employed in this and subsequent samples. Summaries of statistical data occur on pages 550-551, 598-599.

PLATE I.—A view in the experimental sugar orchard, Randolph, Vt. The covers shown above were not of the type used in the experimental work. (See page 344).

PLATE II.—Exterior view of field laboratory, located at the end of the "sugar house." (See page 3.99).

97.16% sucrose and 1.44% invert sugar. The color was 11, flavor 2, and score 700; depreciation from control, color 5, flavor 1, and score 175.

6. Number 6 was a control having exactly the same history as number 1, except that it was concentrated over a brisk fire under conditions which required less than 2 hours to reduce it to sirup. The sirup contained 95.41% sucrose and 0.97% invert sugar. The color was 6, flavor 1, and score 875.

SERIES 2: SIRUPS 7 TO 12

Following the run of March 24 no more sap was obtained until March 27, on which date a light run occurred and enough sap was secured for seven samples. Six of these were inoculated and handled under conditions similar to those described for series 1. The air temperature maintained during the incubation may be seen by referring to the graph (fig. 3). The seventh

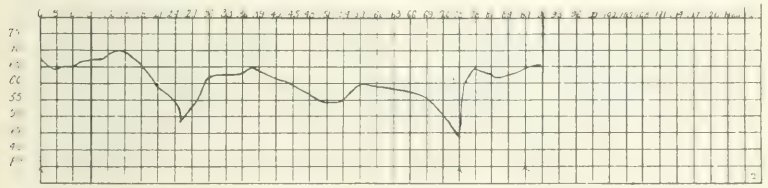

Fig. 3. Graph of incubation temperatures for series 2; saps 7 to 12 inclusive. The arrow heads on next to the bottom line from left to right indicate respectively the time of inoculation, the time of evaporation of the first sample and the time of evaporation of the last sample.

sample was reserved for a control, but, unfortunately, the glass jar containing the sirup was broken during sterilization and the sample was lost. From the notes made at the time of boiling it seems certain that the sirup was at least equal in quality to number 6 in the preceding series and probably slightly superior to it. The sirups of series 2 have therefore been referred to number 6 as a control. One of the saps of this series was titrated in cold solution against phenolphthalein with N/100 sodium hydroxid just before evaporation. The reaction is stated in percent of hundredth normal acid.

7. Number 7 was inoculated with fluorescent organism LVI. It developed typical green souring. The sirup contained 96.16% sucrose and 1.64% invert sugar. The color was 10, flavor 2, and score 750; depreciation from control, color 4, flavor 1, and score 150.

8. Number 8 was inoculated with fluorescent organism 5. A typical green sour sap developed. The sirup contained 97.14% sucrose and 1.15% invert sugar. The color was 10, flavor 2, and score 725; depreciation from control, color 4, flavor 1, and score 150.

9. Number 9 was inoculated with non-fluorescent organism XXVI. It developed a milky type of souring. The sirup contained 95.61% sucrose and 0.78% invert sugar. The color was 5, flavor 2, and score 850. The color was 1 point higher than the control, with a depreciation in flavor of 1, and in total score of 25.

10. Number 10 was inoculated with non-fluorescent organism XXX. It developed a very pronounced milky type of souring, accompanied by an unpleasant odor. The sirup contained 95.86% sucrose and 0.77% invert sugar. The color was 6, flavor 3, and score 750; depreciation from control, color 0, flavor 2, and score 125.

11. Number 11 was inoculated with *Bacillus aceris*, strain LXXXVII, obtained from stringy sap. The sample developed a deep milky color and a yeasty odor and became stringy. The reaction was 170% N/100 acid. The vapor evolved during the concentration was extremely unpleasant, and was sufficiently noticeable to attract the attention of a sugar maker who was passing the house where the field laboratory was established. Although he was entirely unaware of the nature of the experiments, the steam borne to him through an open window elicited the remark, "It smells like the last of sugaring." The sirup contained 96.12% sucrose and 1.72% invert sugar. The color was 7, flavor 4, and score 600; depreciation from control, color 1, flavor 3, and score 275.

12. Number 12 was inoculated with non-fluorescent organism 1, obtained from stringy peas. A milky type of souring developed. The sirup contained 95.71% sucrose and 2.23% invert sugar. The color was 6, flavor 3, and score 750; depreciation from control, color 0, flavor 2, and score 125.

SERIES 3: SIRUPS 13 TO 20

The sap for this series was secured April 1 and handled in the manner described for the two preceding series. The temperature of incubation may be seen by referring to the graph (fig. 4).

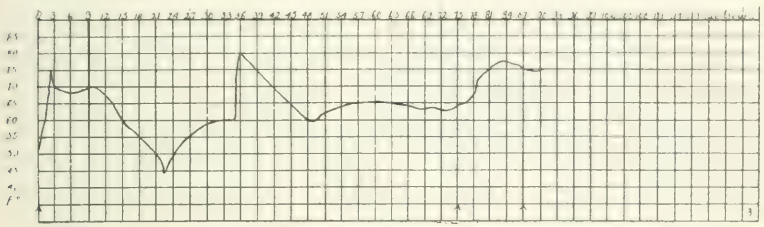

Fig. 4. Graph of incubation temperatures for series 3; saps 13 to 20 inclusive. The arrow heads on next to the bottom line from left to right indicate respectively the time of inoculation, the time of evaporation of the first sample and the time of evaporation of the last sample.

13. Number 13 was inoculated with gray yeast 24. A mixture of organisms developed. Apparently the fluorescent bacteria naturally present in the sap were not held in check by the yeasts, and the product seemed to be the results of the combined action of yeasts and bacteria. The sirup contained 93.34% sucrose and 2.53% invert sugar. The color was 7, flavor 2, and score 800; depreciation from control, color 1, flavor 1, and score 75.

14. Number 14 was inoculated with red yeast 25. A mixture of organisms developed as was the case with the previous sample. The reaction was 9% N/100 acid. The sirup contained 95.30% sucrose and 3.01% invert sugar. The color was 10, flavor 2, and score 725; depreciation from control, color 1, flavor 1, and score 150.

15. Number 15 was inoculated with red yeast LXII and incubated for 3 days. As with other samples previously reported where yeasts were employed a mixture of organisms developed. The reaction was 5% N/100 acid. The sirup contained 93.26% sucrose and 2.98% invert sugar. The color was 8, flavor 2, and score 775; depreciation from control, color 2, flavor 1, and score 100.

16. Number 16 was inoculated with green mold LXIV. The bacteria naturally present developed in conjunction with the mold and the sample had the appearance characteristic of the green type of souring, but it was evident from the tufts of mycelium present that the mold had made good growth. The reaction was 8.2% N/100 acid. The sirup contained 95.73% sucrose and 2.61% invert sugar. The color was 10, flavor 4, and score 525; depreciation from control, color 4, flavor 3, and score 350.

17. Number 17 was inoculated with green mold LXV. A mixed infection resulted. The reaction was 8% N/100 acid. The sirup contained 92.76% sucrose and 3.47% invert sugar. The color was 10, flavor 2, and score 725; depreciation from control, color 4, flavor 1, and score 150.

18. Number 18 was inoculated with green mold LXXIX. A mixed infection resulted. The reaction was 12% N/100 acid. The sirup contained 91.65% sucrose and 3.12% invert sugar. The color was 10, flavor 2, and score 725; depreciation from control, color 4, flavor 1, and score 150.

19. Number 19 was inoculated with a composite culture consisting of fluorescent bacteria, yeasts and molds. The reaction was 5% N/100 acid. The sirup contained 93.28% sucrose and 4.09% invert sugar. The color was 9, flavor 3, and score 675; depreciation from control, color 3, flavor 2, and score 200.

20. Number 20 was held without inoculation at low temperature during the incubation period and reserved as a control. The reaction was 2% N/100 acid. The sirup contained 95.37%

sucrose and 1.58% invert sugar. The color was 6, flavor 1, and score 875.

SIRUPS FROM LAST RUN SAP IN 1909

No more inoculation work was conducted during the season of 1909, but several samples of sirup were made with a view of determining the possible influence of physiological changes in the tree upon the quality of sirup. As previously stated, trees were selected for this purpose which were "running sour," that is to say trees from which clear sap was no longer being obtained. The spouts usually "dry up" or cease to run quite promptly after the appearance of this phenomenon. The aim was to wait till the last day a tree was likely to run before making the experiment. A fresh tap from which the sap flowed clear was then made about 4 inches to one side of the old one. The two types of sap thus secured from the same tree at the same time would presumably be very similar in character until they left the vascular tissues. That from the old taphole, however, becomes very heavily infected as it flows from the spout while the other is relatively free from infection. Any difference in quality of the two sirups obtained under such circumstances would seem to be attributable to the influence of micro-organisms, while any differences in quality between controls of the first runs, and the last run material from the fresh taps, would reasonably be ascribed to physiological changes which had taken place within the tree.

21. Number 21 was made from sweet sap obtained by re-tapping the first sour tree observed during the season. This was drawn on April 12. The sirup contained 95.19% sucrose and 0.82% invert sugar. The color was 3, flavor 1, and score 950. The color was 3 grades superior to the first run control of the season, the flavor equal, and the total score 75 points higher.

22. Number 22 was made from sour sap obtained from the same tree at the same time as the sap for 21. The sap was

only slightly cloudy and had all run the day it was concentrated. The sirup contained 95.27% sucrose and 2.76% invert sugar. The color was 8, flavor 3, and score 700; depreciation from control (number 21), color 5, flavor 2, and score 250. Depreciation from first run control, color 2, flavor 2, and score 175.

23. Number 23 was made from sour sap obtained April 12 and held 24 hours. The sirup contained 92.56% sucrose and 3.55% invert sugar. The color was 14, flavor 3, and score 550; depreciation from control (number 24), color 7, flavor 2, and score 300. Depreciation from first run control, color 8, flavor 2, and score 325.

24. Number 24 was made from sap obtained at the same time and from the same tree as that for number 23. It was drawn from a fresh tap, but was held for twenty-four hours before concentration. The sirup contained 96.02% sucrose and 0.61% invert sugar. The color was 7, flavor 1, and score 850. Depreciation from first run control, color 1, flavor 0, and score 25.

25. Number 25 was made from sour sap which flowed April 16. The sirup contained 94.05% sucrose and 3.13% invert sugar. The color was 14, flavor 3, and score 550; depreciation from control (number 26), color 9, flavor 2, and score 350. Depreciation from first run control, color 8, flavor 2, and score 325.

26. Number 26 was obtained from the same tree as number 25 and at the same time, but from a fresh tap-hole. The samples were concentrated the same day they flowed. The sirup contained 96.42% sucrose and 0.51% invert sugar. The color was 5, flavor 1, and score 900. The color was one grade superior to the first run control, the flavor equal, and the total score 25 points higher.

The results of this single season's experiments taken alone would seem to indicate that the color and flavor of sirup are not impaired by physiological changes in the tree occurring during the sugar season. It is important to note, however, that

the season of 1909 was peculiar. There were no warm periods of importance and the interval between the first run and the last was only three weeks. The commercial season was even shorter since the experimental trees were tapped before the real season opened and most sugar makers in the vicinity gathered their buckets before April 16.

Inoculation Experiments in 1910

The experience gained in 1909 emphasized the importance of more carefully controlled inoculation experiments. To facilitate the progress of the studies it seemed best to establish a laboratory where the more simple bacteriological technique could be carried out in close proximity with the woods. Accordingly, before the opening of the sugar season in 1910, a small rough wooden building was put up in the sugar woods for this purpose. (See Plate II). The floor space available was about 6 by 24 ft. The equipment consisted of an old fashioned box-stove, the top of which could be easily removed to be replaced by the evaporating pan, a blue-flame oil stove with an oven, an Arnold steam sterilizer, a microscope, Petri dishes, test tubes and other glassware, a few chemicals and the necessary sugar tools. (See Plate III). Since the laboratory was of rough construction and consequently became cold quickly, it was necessary to provide some apparatus in which the inoculated sap could be stored, in order to maintain a reasonably constant temperature during the period of incubation. For this purpose a rather crude modification of the modern fireless cooker was provided. (See Plates III and IV). This consisted of a box the approximate interior dimensions of which were, height 20 inches, width 40 inches, and length 16 feet. The walls were made of 2 double layers of matched sheathing between which was an air space of 1 inch. Building paper was used between each of the double layers of sheathing and on each side of the one inch air space. The top, bottom, and sides were of similar construction, the cover being so arranged as to break joints as

is customary with the doors of safes or refrigerators. The cover was formed in sections so as to permit opening one end without admitting cold air to the entire contents.

It was the first intention to maintain the temperature by introducing hot freestones into the incubator when the temperature of the sap began to fall. It soon appeared, however, during the extremely cold weather encountered shortly after starting the first series of the year, that this means of control was inadequate and the more satisfactory method of introducing buckets of boiling water was resorted to. This raised the air temperature in the incubator very promptly and the heat was gradually imparted to the sap. The temperature of the samples was occasionally tested by introducing a sterile thermometer into the buckets, and whenever necessary additional heat was supplied.

A self recording thermometer was placed in the incubator from the tracings of which the temperature curves here reproduced were obtained. It should be borne in mind, however, that these curves by no means represent the temperature of the sap which was far more constant than that of the air. It never fell as low nor did it rise as high. The graphs are of value, however, in that they give some indication of the conditions of incubation.

The material was incubated in new tin buckets which were thoroughly scalded immediately before filling. They had a capacity of 20 quarts and were provided with covers. Inoculation was made with a 70 cc. young culture of the specific organism employed. The controls were treated with an equivalent amount of the sterile medium which consisted of 65 cc. of sap and 5 cc. of nutrient bouillon.

Before being made into sirup each sap was thoroughly mixed and a small sample was withdrawn and sterilized to be preserved for the purposes of another investigation, the results of which it is expected will eventually be published by the station chemist. As a rule the reaction was determined by titrating against

phenolphthalein in the cold with N/100 sodium hydroxid, and a bacteriological analysis was made in order to determine the degree of success which had been attained in inoculation. The sap was then placed in a thoroughly clean pan over a brisk, flaming wood fire and evaporated to a volume of about 1 quart, when it was transferred to a white agate ware basin and concentrated to a density of 11 pounds to the gallon as indicated by the thermometer. It was the custom to determine daily the temperature at which water boiled in the laboratory, and to subtract from 219 the difference between 212 and the boiling point found, in order to arrive at the temperature at which evaporation should be discontinued during that day. Provision was thus made for fluctuations in the boiling point due to atmospheric conditions.

The sugar season of 1910 opened unusually early, before the laboratory was quite ready for use. The first run occurred on March 3 and 4, but the experimental trees were not tapped until March 6.

SERIES 4: SIRUPS 27 TO 40

The sap for this series was collected March 7, and placed in the incubating buckets in 16 quart portions. It was not so thoroughly mixed as to insure absolutely uniform chemical composition, but was handled in a manner to secure approximate uniformity. The temperature of all except two of the samples was raised to 40° C. before placing them in the incubator. One of these was allowed to stand outside where a temperature of 0° to 10° C. prevailed, while the other was heated to 80° C. and placed in the incubator. These two samples, together with one of those heated at 40° C., constituted the controls. When the temperature of the sap had dropped to 28° C. the samples were inoculated with their respective organisms and incubated for 3 days. Evaporation of the samples began at 9 a. m., March 11, under conditions which made it possible to average the production of a sirup in an hour and a quarter. The temperature of incubator may be seen by reference to figure 5.

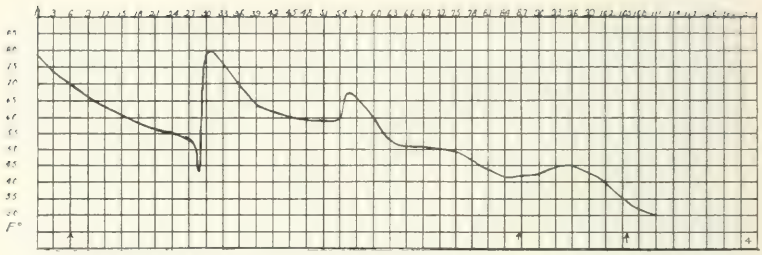

Fig. 5. Graph of incubation temperatures for series 4 ; saps 27 to 40 inclusive. The arrow heads on next to the bottom line from left to right indicate respectively the time of inoculation, the time of evaporation of the first sample and the time of evaporation of the last sample.

27. Number 27 was the control kept outside the incubator at a temperature only slightly above its freezing point. Notwithstanding the low temperature it contained 10,000 organisms per cc. although there was no evidence of clouding at the time of evaporation. The sirup contained 96.19% sucrose and 0.30% invert sugar. The color was 4, flavor 2, and score 875.

28. Number 28 was inoculated with gray yeast 24. The sap became cloudy, at first greenish, then milky. The organism was recovered in mixed culture. The plates showed 437,000 colonies per cc. The sirup contained 82.83% sucrose and 12.15% invert sugar. The color was 11, flavor 4, and score 500; depreciation from control, color 7, flavor 2, and score 375.

29. Number 29 was inoculated with fluorescent organism CXXXIX. A green type of souring developed, the sap becoming cloudy within twelve hours. The organism was recovered in practically pure culture, the plates showing 12,330,000 per cc. The sirup contained 94.19% sucrose and 0.38% invert sugar. The color was 8, flavor 2^1, and score 750; depreciation from control, color 4, flavor 0^1, and score 125.

30. Number 30 was inoculated with fluorescent organism CLIX. It was recovered apparently in pure culture from the green sour sap which resulted. The plates showed 3,262,000 colonies per cc. The sirup contained 93.75% sucrose and 0.89% invert sugar. The color was 8, flavor 2, and score 775; depreciation from control, color 4, flavor 0, and score 100.

31. Number 31 was inoculated with gray yeast CXXI. The organism was not recovered, but the plates showed a count of 5,667,000 colonies per cc., in which those of the fluorescent group greatly predominated. The sirup contained 96.62% sucrose and 0.30% invert sugar. The color was 6, flavor 2 and score 825; depreciation from control, color 2, flavor 0, and score 50.

32. Number 32 was inoculated with gray yeast CLXXX. The organism was not recovered. The plates showed a count of 5,000,000 colonies per cc., most of which were of the green fluorescent type. The sirup contained 95.91% sucrose and 0.46% invert sugar. The color was 6, flavor 2, and score 825; depreciation from control, color 2, flavor 0, and score 50.

33. Number 33 was inoculated with gray yeast CLXVIII. The organism was not recovered. The plates showed 1,950,000 colonies per cc., most of them characteristic of the fluorescent organisms. The sirup contained 95.61% sucrose and 0.23% invert sugar. The color was 7, flavor 2, and score 800, depreciation from control, color 3, flavor 0, and score 75.

34. Number 34 was the control previously mentioned as having been heated to 40° C., and placed in the incubator. As would be expected the sap promptly clouded, developing a green type of souring. The plates showed a count of 7,312,000 organisms per cc. Fluorescence was pronounced. The sirup contained 95.86% sucrose and 0.42% invert sugar. The color was 6, flavor 2^1, and score 800; depreciation from cold control, color 2, flavor 0^1, and score 75.

35. Number 35 was inoculated with red yeast CXI. Deep clouding developed and the organism was recovered in association with large numbers of fluorescent bacteria. The plates showed a count of 2,255,000 organisms per cc. The sirup contained 95.13% sucrose and 1.15% invert sugar. The color was 12, flavor 2^1, and score 650; depreciation from control, color 8, flavor 0^1, and score 225.

36. Number 36 was inoculated with red yeast CII. The sap clouded promptly but only a very few colonies of the organism were recovered. The plates showed a count of 7,312,000 in which the fluorescent group predominated. The sirup contained 96.16% sucrose and 0.61% invert sugar. The color was 9, flavor 2^1, and score 725; depreciation from control, color 5, flavor 0^1, and score 150.

37. Number 37 was inoculated with red yeast CLXXV. The organism was not recovered. The plates gave a count of 8,825,000 per cc. with pronounced fluorescence. The sirup contained 95.88% sucrose and 0.64% invert sugar. The color was 11, flavor 2^1, and score 675; depreciation from control, color 7, flavor 0^1, and score 200.

38. Number 38 was the control placed in the incubator after heating to 80° C. The plates showed a count of 162,500 colonies per cc. of which very few developed fluorescence. The sirup contained 94.80% sucrose and 0.48% invert sugar. The color was 5, flavor 2^1, and score 825; depreciation from cold control, color 1, flavor 0^1, and score 50.

39. Number 39 was inoculated with gray yeast C. Green souring developed and the organism was not recovered. The plates showed a count of 9,750,000 colonies per cc. with pronounced fluorescence. The sirup contained 96.18% sucrose and 0.26% invert sugar. The color was 7, flavor 2, and score 800; depreciation from control, color 3, flavor 0, and score 75.

40. Number 40 was a control placed in the incubator without heating. Plates showed a count of 5,850,000 organisms with the fluorescent group in predominance. The sirup contained 95.05% sucrose and 0.64% invert sugar. The color was 9, flavor 2, and score 750; depreciation from cold control, color 5, flavor 0, and score 125.

The results of this series confirmed the suspicion entertained from the inoculation trials of the previous season's work, that the yeasts did not develop readily in maple sap, but that the bacteria normally present gained the ascendency. The changes in

the sirups of this series, with the exception of number 28, seemed to have been very largely produced by the fluorescent group of organisms. Even the cold control was apparently considerably injured both in color and flavor by the organisms developing in it during the incubation period, so that the depreciation in quality due to their action is probably greater than is indicated by the figures given above.

That this supposition is well grounded is shown by the results obtained in later series.

SERIES 5: SIRUPS 41 TO 48

Following the run obtained on March 7, there was a freeze and no considerable amount of sap was obtained again until March 14. The sap was gathered with a team and a sufficient quantity for 8 samples was withdrawn as soon as it reached the sugar house. Six of these were heated to boiling and placed in the incubating buckets while two were left unheated, their temperature being very near 0° C. On the morning of March 15, five of the heated samples were inoculated and allowed to incubate for 3 days at temperatures indicated in the graph (figure

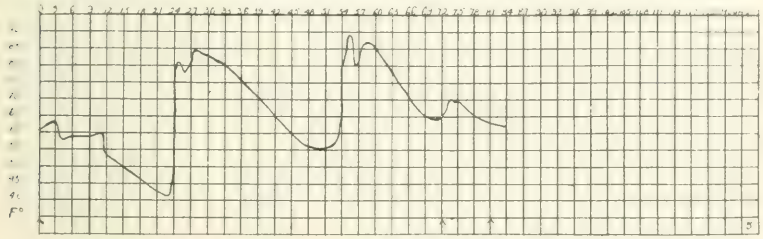

Fig. 6. Graph of incubation temperatures for series 5; saps 41 to 48 inclusive. The arrow heads on next to the bottom line from left to right indicate respectively the time of inoculation, the time of evaporation of the first sample and the time of evaporation of the last sample.

6). Before evaporation the samples were titrated, plated, and sampled as described for the previous series.

41 Number 41 was a control left standing over night before being made into sirup on the morning of March 15 when it contained 66 organisms per cc. and reacted 1% N/100 acid.

The sirup contained 97.82% sucrose and 0.45% invert sugar. The color was 4, flavor 1, and score 925.

42. Number 42 was inoculated with red yeast CVII. A milky appearance developed and the organism was recovered in considerable quantity in association with bacteria producing non-fluorescent colonies. The plates gave a count of 13,650,000 colonies per cc. The reaction was 19% N/100 acid. The sirup contained 93.27% sucrose, and 2.14% invert sugar. The color was 5, flavor 2^2, and score 800; depreciation from control, color 1, flavor 1^2, and score 125.

43. Number 43 was a control heated to boiling and incubated for 3 days. The material became cloudy the third day and the plates showed a development of 1,625,000 organisms per cc., mostly of the non-fluorescent type. The reaction was 6.5% N/100 acid. The sirup contained 94.19% sucrose and 2.64% invert sugar. The color was 7, flavor 2, and score 800; depreciation from cold control, color 3, flavor 1, and score 125.

44. Number 44 was inoculated with fluorescent organism CXLVIII. A greenish type of clouding developed and the organism was recovered in numbers amounting to 9,000,000 per cc. The reaction was 10% N/100 acid. The sirup contained 93.03% sucrose and 1.40% invert sugar. The color was 9, flavor 3, and score 675; depreciation from control, color 5, flavor 2, and score 250.

45. Number 45 was a control incubated for 3 days in the cold. The sap remained perfectly clear. The plates revealed a bacterial content of 195,000 per cc. The reaction was 4.5% N/100 acid. The sirup contained 96.28% sucrose and 0.51% invert sugar. The color was 4, flavor 1, and score 925; depreciation from control (number 41), color 0, flavor 0, and score 0.

46. Number 46 was inoculated with pink coccus CIV. Pronounced clouding of the milky type and a yeasty odor developed. The plates showed a count of 14,300,000 organisms per cc. among which the introduced organism was predominant. The reaction was 10% N/100 acid. The sirup contained

95.07% sucrose and 1.05% invert sugar. The color was 6, flavor 2, and score 825; depreciation from control, color 2, flavor 1, and score 100.

47. Number 47 was inoculated with pink coccus IV. A milky type of souring developed. The plates showed a count of 1,950,000 organisms per cc., the majority of which apparently were produced by the introduced organism. The reaction was 9.6% N/100 acid. The sirup contained 88.81% sucrose and 7.90% invert sugar. The color was 6, flavor 3, and score 750; depreciation from control, color 2, flavor 2, and score 175.

48. Number 48 was inoculated with gray yeast CLXXX. The sap became milky and developed a yeasty odor. The organism was recovered in association with bacteria the colonies of which resembled those of the subtilis group. The plates gave a total count of 1,625,000 per cc. The reaction was 10.7% N/100 acid. The sirup contained 93.89% sucrose and 2.35% invert sugar. The color was 6, flavor 2^2, and score 775; depreciation from control, color 2, flavor 1^2, and score 150.

SERIES 6: SIRUPS 49 TO 57

Series 6 was made from sap which flowed on March 20. A sufficient quantity was gathered at four o'clock for 9 samples and the material was thoroughly mixed to insure uniformity of chemical composition. Two of the samples were left untreated while the others were heated to boiling, cooled, and, with the exception of one, inoculated. The air temperature maintained during the 3 days' incubation is shown in the accompanying graph (figure 7).

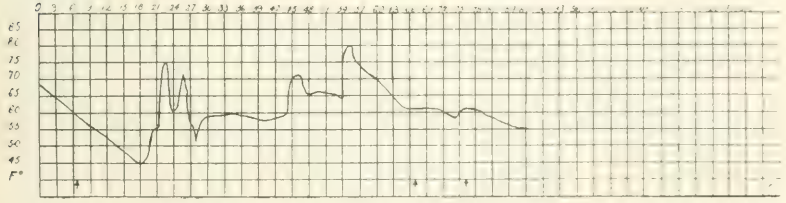

Fig. 7. Graph of incubation temperatures for series 6; saps 49 to 57 inclusive. The arrow heads on next to the bottom line from left to right indicate respectively the time of inoculation, the time of evaporation of the first sample and the time of evaporation of the last sample.

49. Number 49 was a control evaporated immediately after gathering. A bacteriological analysis showed a count of only 100 organisms per cc. The reaction was 1% N/100 acid. The sirup contained 96.51% sucrose and 0.42% invert sugar. The color was 2, flavor 1, and score 975. This sirup was considered the best specimen obtained during the entire period covered by the work.

50. Number 50 was inoculated with pink coccus XLIX. Deep clouding resulted and the coccus was recovered in practically pure culture. The plates showed a count of 1,950,000 organisms per cc. The reaction was 8% N/100 acid. The sirup contained 93.83% sucrose and 2.20% invert sugar. The color was 6, flavor 2^1, and score 800; depreciation from control, color 4, flavor 1^1 and score 175.

51. Number 51 was inoculated with pink coccus CVI. The sap became deeply clouded. The organism was recovered, the plates showing a count of 4,225,000 per cc. with the introduced species in predominance. The reaction was 3.5% N/100 acid. The sirup contained 97.30% sucrose and 0.65% invert sugar. The color was 4, flavor 2, and score 875; depreciation from control, color 2, flavor 1, and score 100.

52. Number 52 was inoculated with gray yeast CLXII. A milky white clouding developed. The organism was recovered in mixed culture. The plates showed a count of 1,275,000 colonies per cc. About 30,000 of these were characteristic of the introduced organism, while most of the others were bacterial colonies characteristic of the subtilis group. The sirup contained 96.84% sucrose and 1.21% invert sugar. The color was 5, flavor 2, and score 850; depreciation from control, color 3, flavor 1, and score 125.

53. Number 53 was inoculated with gray yeast CLXXI. A milky type of souring developed. The organism was recovered in association with bacteria of the subtilis and fluorescent groups. The plates showed a count of 630,000 organisms per cc. The reaction was 6% N/100 acid. The sirup contained 92.13%

sucrose and 3.96% invert sugar. The color was 6, flavor 3, and score 750; depreciation from control, color 4, flavor 2, and score 225.

54. Number 54 was inoculated with pink yeast CXXVI. A deep milky appearance developed to a pronounced degree and an unpleasant yeasty odor was noted. The organism was recovered, the plates showing a count of 1,300,000 organisms per cc. The reaction was 8.5% N/100 acid. The sirup contained 94.11% sucrose, and 2.71% invert sugar. The color was 7, flavor 2^2, and score 750; depreciation from control, color 5, flavor 1^2, and score 225.

55. Number 55 was inoculated with a non-fluorescent organism CVIII. A milky type of souring developed in which a brownish color was evident in strong light, and a peculiar bacterial odor was noted. The organism was recovered in practically pure culture. The plates showed a total count of 7,230,000 organisms per cc. The reaction was 6% N/100 acid. The sirup contained 94.76% sucrose and 2.38% invert sugar. The color was 5, flavor 2, and score 850; depreciation from control, color 3, flavor 1, and score 125.

56. Number 56 was the incubator control heated to boiling and placed in the incubator with the other samples without inoculation. It clouded slightly during the incubation period. The plates poured revealed a bacterial count of 320,000 per cc. The reaction was 3.1% N/100 acid. The sirup contained 96.02% sucrose and 1.35% invert sugar. The color was 5, flavor 2, and score 850; depreciation from control, color 3, flavor 1, and score 125.

57. Number 57 was the control not heated and retained outside the incubator at low temperature during the incubation period. At the time of evaporation it had a temperature of 10° C. The plates showed a development of 37,000 bacteria per cc. The reaction was 2.5% N/100 acid. The sirup contained 96.47% sucrose and 0.60% invert sugar. The color was 2, flavor 1, and score 975; depreciation from control (number

491, color 0, flavor 0, and score 6. While this sample was given the same score as number 49, it was considered by the expert who scored the samples to be very slightly its inferior.

SERIES 7: SIRUPS 58 TO 65

The sap for this series ran on March 21. It was collected with the general supply in a gathering tub late in the evening, strained into buckets and allowed to remain over night in a cold place. On the morning of the following day all but one of the samples was heated to boiling and cooled at once by placing the buckets in ice water. As soon as cool the material was placed in the incubator and cultures of organisms were added to the samples designed for inoculation. The air temperature maintained during the incubation period appears on the accompanying graph (figure 8).

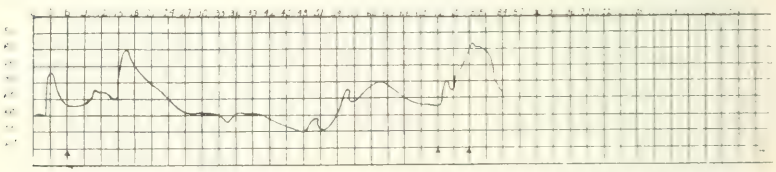

Fig. 8. Graph of incubation temperatures for series 7; saps 58 to 65 inclusive. The arrow heads on next to the bottom line from left to right indicate respectively the time of inoculation, the time of evaporation of the first sample and the time of evaporation of the last sample.

58. Number 58 was a control evaporated on the morning of March 22. The plates developed 380 colonies per cc., among which those of the fluorescent bacteria predominated. The reaction was 3% N/100 acid. The sirup contained 97.01% sucrose and 0.43% invert sugar. The color was 3, flavor 1, and score 950. This sample was regarded by the judge as the third best sirup obtained during the entire series of experiments.

59. Number 59 was inoculated with red yeast CXXIII. A brownish-white clouding resulted. The organism was recovered in association with spore-bearing bacteria apparently belonging to the subtilis group. The plates showed a development of 325,000 colonies per cc. The reaction was 8% N/100 acid. The sirup contained 94.74% sucrose and 2.03% invert sugar. The color

was 7, flavor 3, and score 725; depreciation from control, color 4, flavor 2, and score 225.

60. Number 60 was inoculated with red yeast CXIII. A milky brown souring developed. The organism was recovered in association with bacteria belonging to the fluorescent and subtilis groups. The count showed 1,950,000 colonies per cc. The reaction was 8.4% N/100 acid. The sirup contained 95.44% sucrose and 1.76% invert sugar. The color was 6, flavor 3, and score 750; depreciation from control, color 3, flavor 2, and score 200.

61. Number 61 was inoculated with red yeast CX. A reddish-brown clouding developed. The organism was recovered, the plates showing a count of 650,000 colonies per cc. among which those of the introduced organism predominated. The reaction was 5.5% N/100 acid. The sirup contained 95.80% sucrose and 1.77% invert sugar. The color was 9, flavor 3, and score 675; depreciation from control, color 6, flavor 2, and score 275.

62. Number 62 was inoculated with red yeast 25. A reddish clouding developed accompanied by a sour yeasty odor. The organism was recovered in association with bacteria, 650,000 colonies per cc. being developed of which practically one third were those of the introduced organism. The reaction was 9.5% N/100 acid. The sirup contained 89.27% sucrose and 7.30% invert sugar. The color was 9, flavor 4, and score 550; depreciation from control, color 6, flavor 3, and score 400.

63. Number 63 was inoculated with red yeast CXIV. The sap became cloudy with a reddish-brown hue. Out of the 975,000 colonies per cc. which developed on the plates, 175,000 were characteristic of the introduced organism. The reaction was 11% N/100 acid. The sirup contained 89.06% sucrose and 7.63% invert sugar. The color was 8, flavor 3, and score 700; depreciation from control, color 5, flavor 2, and score 250.

64. Number 64 was inoculated with red yeast CXX. A sour yeasty odor developed and the sap became deeply clouded with a reddish cast. The organism was recovered. The plates

showed a development of 97,500 colonies per cc., most of which were characteristic of the introduced organism. The reaction was 10% N/100 acid. The sirup contained 91.64% sucrose and 5.86% invert sugar. The color was 8, flavor 3, and score 700; depreciation from control; color 5, flavor 2, and score 250.

65. Number 65 was a control retained in the incubator. The sap clouded early and appeared like the inoculated samples. The plates showed a development of 1,950,000 colonies of various species. The reaction was 9% N/100 acid. The sirup contained 91.89% sucrose and 3.52% invert sugar. The color was 6, flavor 4, and score 625; depreciation from control (number 58), color 3, flavor 3, and score 325.

INFLUENCE OF THE CONTAINER UPON THE QUALITY OF THE SIRUP

Under the above heading will be discussed the sirups made from the saps previously referred to in connection with the discussion of the influence of the container upon the bacterial content of the sap (page 343). It will be remembered that six trees were employed which had been tapped with special care and upon which tin buckets were hung. For the purpose of a part of this experiment, they were replaced by wooden ones. The tin buckets were kept free from the accumulation of micro-organisms by frequent washings and scaldings.

66. Number 66 was made from sap collected March 21 and 22, in clean tin buckets. The composite sample from the 6 trees contained 140 organisms per cc., most of them of a single type previously mentioned as characteristic of tree 3 (Plate VI). The reaction was 2% N/100 acid. The sirup contained 96.62% sucrose and 0.41% invert sugar. The color was 3, flavor 1, and score 950.

67. Number 67 was made from sap obtained from the same trees as was number 66 but it flowed two days later on March 23 and 24. The tin buckets were replaced by wooden buckets formerly employed in the sugar place where the work was done. They had been soaked out in the usual way and thoroughly

washed before being hung on the trees. That the crevices and interstices of the wood afforded ample lodging places for micro-organisms is readily seen from the fact that 6,500,000 colonies per cc. developed from the mixture of sap obtained, and only a few of them were characteristic of the organism from tree 3 (Plate VII). The reaction was 5.5% N/100 acid. The sirup contained 88.25% sucrose and 6.21% invert sugar. The color was 9, flavor 4, and score 550; depreciation from control (number 66), color 6, flavor 3, and score 400.

SERIES 8: SIRUPS 68 TO 73

The sap of this series ran on March 22, and was collected in the evening and stored over night in buckets, at a temperature slightly above its freezing point. The following morning the samples were thoroughly mixed, and all but one were heated to boiling and allowed to cool in water. At 3 p. m., they were placed in the incubator and inoculated. The temperature maintained during the three days' incubation period may be seen by reference to the graph (figure 9).

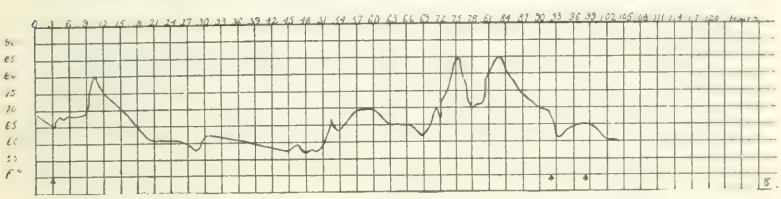

Fig. 9. Graph of incubation temperatures for series 8; saps 68 to 73 inclusive. The arrow heads on next to the bottom line from left to right indicate respectively the time of inoculation, the time of evaporation of the first sample and the time of evaporation of the last sample.

68. Number 68 was a control boiled down on the morning of March 23. Just before evaporation it contained 500 organisms per cc. The reaction was 2.5% N/100 acid. The sirup contained 96.90% sucrose and 0.51% invert sugar. The color was 4, flavor 1, and score 925.

69. Number 69 was inoculated with fluorescent organism CLIV. It was recovered in practically pure culture from the green sour sap which resulted. The plates showed a count of

19,500,000 organisms per cc. The reaction was 10% N/100 acid. The sirup contained 93.87% sucrose and 1.59% invert sugar. The color was 9, flavor 3, and score 675; depreciation from control, color 5, flavor 2, and score 250.

70. Number 70 was inoculated with green fluorescent organism CXXXIII. A greenish-brown souring developed promptly and the organism was recovered in practically pure culture. The plates showed a count of 20,400,000 per cc. The reaction was 10% N/100 acid. The sirup contained 93.47% sucrose and 2.46% invert sugar. The color was 11, flavor 4, and score 500; depreciation from control, color 7, flavor 3, and score 425.

71. Number 71 was inoculated with green fluorescent organism CXLVII. A greenish-white clouding appeared within a day. The plates showed a development of 23,400,000 organisms per cc., practically all of which were of a type characteristic of the introduced species. The reaction was 6.5% N/100 acid. The sirup contained 93.69% sucrose and 0.58% invert sugar. The color was 14, flavor 4, and score 425; depreciation from control, color 10, flavor 3, and score 500.

72. Number 72 was inoculated with green fluorescent organism CLII. A green souring occurred. The plates showed a development of 29,250,000 colonies per cc., practically all of the fluorescent type. The reaction was 10% N 100 acid. The sirup contained 95.07% sucrose and 0.87% invert sugar. The color was 13, flavor 4, and score 450. Depreciation from control, color 9, flavor 3, score 475.

73. Number 73 was inoculated with green fluorescent organism CLVIII. The sap promptly clouded, becoming at first greenish and then milky. The plates showed a development of 23,400,000 colonies per cc. with pronounced green fluorescence. The reaction was 2.3% N/100 acid. The sirup contained 97.47% sucrose, and 0.68% invert sugar. The color was 6, flavor 2, and score 825; depreciation from control, color 2, flavor 1, and score 100.

SERIES 9: SIRUPS 74 TO 81

The sap for this series was gathered in pails and brought to the laboratory about 10 p. m., March 24. It was carefully mixed and divided into 18-quart portions, all but one of which were heated to boiling. The following morning the samples which had been heated were placed in the incubator and inoculated, with the exception of one which was reserved as an incubator control. The air temperature maintained during the incubation period may be seen by referring to the accompanying graph (figure 10).

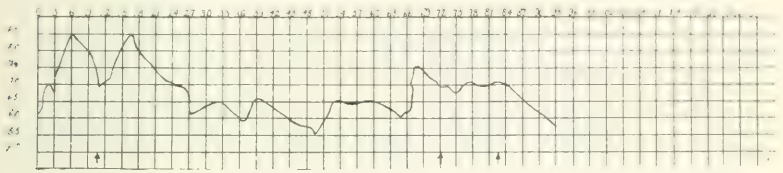

Fig. 10. Graph of incubation temperatures for series 9 : saps 74 to 81 inclusive. The arrow heads on next to the bottom line from left to right indicate respectively the time of inoculation, the time of evaporation of the first sample and the time of evaporation of the last sample.

74. Number 74 was the control which was not heated. It was evaporated on the morning of March 25. The sap contained 750 organisms per cc. The reaction was 3% N/100 acid. Unfortunately the sirup was slightly burned in the pan so that its color and flavor were seriously impaired. For this reason the sirups of this series have been referred to number 68 as a control. Sirup 74 contained 96.36% sucrose and 0.70% invert sugar. The color was 9, flavor 4, and score 550; depreciation from control (number 68), color 5, flavor 3, and score 375.

75. Number 75 was inoculated with fluorescent organism CLXXIX. The plates showed a development of 10,400,000 colonies per cc. divided between those characteristic of the fluorescent group and those of another organism of the subtilis type. The reaction was 10% N/100 acid. The sirup contained 84.74% sucrose and 10.77% invert sugar. The color was 9, flavor 3, and score 675; depreciation from control, color 5, flavor 2, and score 250.

76. Number 76 was inoculated with fluorescent organism CLV. The organism was recovered, but in association with great number of the spore-bearing bacteria noted in connection with the previous sample. The plates showed a count of 4,875,000 organisms per cc. The reaction was 10.7% N/100 acid. The sirup contained 87.03% sucrose and 8.94% invert sugar. The color was 10, flavor 3, and score 650; depreciation from control, color 6, flavor 2, and score 275.

77. Number 77 was inoculated with green fluorescent organism CLXXVII. 11,700,000 colonies per cc. were obtained. These were divided between the two types of organisms mentioned for the preceding samples of this series. The reaction was 10.1% N/100 acid. The sirup contained 88.13% sucrose and 8.24% invert sugar. The color was 8, flavor 3, and score 700; depreciation from control, color 4, flavor 2, and score 225.

78. Number 78 was inoculated with green fluorescent organism XXXVI. Here again two types of colonies were recovered. The total count was 14,625,000 colonies per cc. The reaction was 11.2% N/100 acid. The sirup contained 83.86% sucrose and 10.84% invert sugar. The color was 8, flavor 4, and score 575; depreciation from control, color, 4, flavor 3, and score 350.

79. Number 79 was inoculated with non-fluorescent organism CLXIII which itself belongs to the subtilis group. 6,500,000 colonies per cc. were recovered, practically all of which resembled those of the introduced organism. The reaction was 25.4% N/100 acid. The sirup contained 78.78% sucrose and 15.25% invert sugar. The color was 7, flavor 4, and score 600; depreciation from control, color 3, flavor 3, and score 325.

80. Number 80 was inoculated with pink yeast CLXVIII. The organism was not recovered. 9,750,000 colonies were obtained per cc., all of which were characteristic of the subtilis type. The sirup contained 79.20% sucrose and 16.13% invert sugar.

The color was 10, flavor 4, and score 525; depreciation from control, color 6, flavor 3, and score 400.

81. Number 81 was a control heated to boiling and left in the incubator without inoculation. It developed 3,250,000 colonies per cc., typical of the subtilis group. The reaction was 10% N/100 acid. The sirup contained 89.23% sucrose and 7.51% invert sugar. The color was 7, flavor 4, and score 600; depreciation from control (number 68), color 3, flavor 3, and score 325.

It is evident that the sap used in the above series was heavily contaminated by some spore-bearing organism which apparently has the power of inverting sugar and which exercises a further detrimental influence upon the flavor of sirup. Reference to the laboratory notes shows that after being heated the samples were placed without being covered on the top of instead of within the incubator to cool over night, in order that they might be ready for inoculation the following morning; but to prevent the entrance of falling dust a shelter of new clean paper was supported a few inches above the tops of the buckets. During the night there occurred a heavy wind storm. The ground was partially bare and it is possible that spore laden dust entered the laboratory through the rather large cracks which had opened in the single layer of boards constituting the floor, and that infection was produced in this way. Unfortunately the infection was not suspected until the results of the bacteriological and chemical analyses were known. Cultures of the organism were not secured so that its identity is uncertain, but the agar plate colonies were strikingly similar both in macroscopic and microscopic appearance to those of *Bacillus subtilis* and spore formation was apparent on unstained preparations from 4 days' agar colonies.

SUGAR AND SIRUP FROM SOUR SAP

The inoculation work of the season was completed with the preceding series, but a few experiments were made with natural sour sap.

82. Number 82 was made from sap collected March 29 from two trees located in a sheltered position which received an abundance of sunlight. The sap obtained from these trees naturally soured somewhat earlier than the average sap in the orchard as a whole and was just about ready to stop running at the time this experiment was started. The sap had been allowed to remain without being collected since the first indications of serious souring, 2 or 3 days before. Neither titration nor bacteriological count was made. The sap was concentrated to sugar at once, to see whether it would grain properly, which it did. The sample was neither scored nor analyzed.

83. Number 83 was made from sap collected April 2 and represented all the sap that had flowed from the trees mentioned under 82, since March 29. At the time of collection these trees had apparently entirely ceased running. The sap contained 43,875,000 organisms per cc. The reaction was 6% N/100 acid. Like the preceding sample this was evaporated at once to a sugar which grained readily. It was neither scored nor analyzed.

84. Number 84 was made April 4 from a part of the same sap from which 83 was made. The sirup contained 91.14% sucrose and 2.83% invert sugar. The color was 20, flavor 3[1], and score 350.

85. Number 85 was made from sour sap collected April 2, but which had been accumulating in the buckets for several days. It contained 11,275,000 organisms per cc. The reaction was 6% N/100 acid. The sirup contained 94.68% sucrose and 1.57% invert sugar. The color was darker than 20, flavor 3[1], and score 325.

86. Number 86 had exactly the same history as 85 except that when nearly evaporated to sirup it was allowed to cool to about 35° C., following which the beaten white of two eggs was added in order to test the clarifying power of this treatment. The boiling was then continued. The sirup contained 93.21% sucrose and 1.23% invert sugar. The color was darker than

20 but not quite as dark as that of number 85. The flavor was 3[1], and score 325.

SIRUP FROM LAST RUN SAP IN 1910

The plan pursued in the following experiments was exactly the same as that described for similar experiments the previous year (page 357). The first trees were retapped April 3. It was the intention to continue the experiments upon different trees until the flow of sap was so reduced that it was impossible to secure enough to make even a small sample of sirup. Unfortunately for the purpose of the work the weather became warm and night freezes ceased altogether, so that only a single experiment of this character was possible.

87. Number 87 was secured from the fresh tap-hole of a tree which had yielded sour sap for a considerable number of days and upon which the leaf buds were already opened. The sap contained 5 organisms per cc. The reaction was 2.5% N/100 acid. The sirup contained 97.27% sucrose and 0.26% invert sugar. The color was 4, flavor 5, and score 525; depreciation from first run control, color 0, flavor 3, score 250.

88. Number 88 was made from the same tree as number 87 but from the sap flowing from the old tap-hole. The plates gave a count of 73,125,000 organisms per cc. The reaction was 4% N/100 acid. The sirup contained 94.56% sucrose and 1.79% invert sugar. The color was 11, flavor 6, and score 250; depreciation from control (number 87), color 7, flavor 1, and score 275. Depreciation from first run control, color 7, flavor 4, score 625.

The results afforded by these two samples are interesting and significant, for it will be observed that the flavor of the last run material in 1910 was very seriously impaired, even when bacteria were excluded, the typical buddy flavor being present in an unmistakable degree, while the color remained light. Manifestly the poor flavor of No. 87 can not be attributed to the influence of the bacteria present for they were practically absent, but 5 per cc. being found. Its cause must be sought elsewhere; and naturally

one suspects physiological changes occurring within the tree. These results do not agree with those obtained in 1909, and cited in bulletin 151, as well as on pages 357-358 in this issue; but the contradiction may probably be explained by the divergencies in the prevailing weather conditions during the two seasons. The spring of 1910 was interrupted by periods of warm weather which started the trees into vegetative activity at least two or three weeks before the close of the sugar season, whereas the season of 1909 was short and exhibited no warm periods intervening with cold spells.

The small amount of invert sugar present in No. 87 confirms the suggestion obtained from the results of 1909, that the invert sugar content of a sap tends to decrease rather than increase as the season advances.

INOCULATION EXPERIMENTS IN 1911

The considerable number of instances in which the plates from incubated samples failed to return approximately pure cultures of the introduced organism in 1910, led to a modification of the procedure for 1911. The incubation buckets were discarded and a large number of 2 quart glass preserving jars were secured in which the sap was placed and subjected to fractional sterilization before inoculation. The covers were left on the jars but were not clamped down, so that the protection from infection was approximately the same as is afforded in Petri dish cultures. For purposes of sterilization, the jars were placed in a steam chamber and treated as is customary in sterilization with flowing steam at atmospheric pressure. Except in the last series of the season the sap was subjected to two sterilizations only. The last series was steamed on each of three consecutive days. This change was made because one of the jars of the control of the preceding series developed cloudiness, which was found upon examination to be due to spore-bearing organisms of the subtilis type. As in the preceding season, the success of the inoculation experiments was controlled by bacteriological examination in

which the poured plate method was employed, but aliquots were not used and no count of the number of recovered organisms was obtained.

The saps were mixed and titrated immediately before concentration, plates were poured to determine the character of the infection, and a sample was removed and sterilized for the purpose of the experiment previously mentioned. The sirups were handled in all respects like those of the preceding year.

SERIES 10: SIRUPS 89 TO 96

The sap for this series flowed on March 22 and was gathered and given the first sterilization late in the afternoon of that day. The second sterilization followed after an interval of 24 hours. The samples were inoculated as soon as they were sufficiently cool, and were incubated for 3½ days. The air temperature maintained in the incubator is shown in the accompanying graph (figure 11).

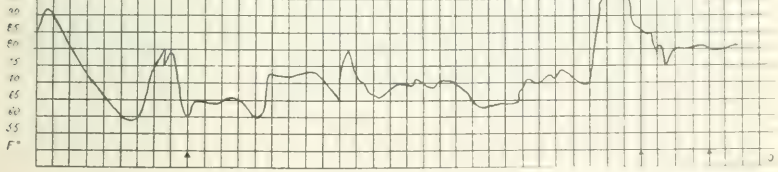

Fig. 11. Graph of incubation temperatures for series 10; saps 89 to 96 inclusive. The arrow heads on next to the bottom line from left to right indicate respectively the time of inoculation, the time of evaporation of the first sample and the time of evaporation of the last sample.

89. Number 89 was a control boiled down the day it was obtained from the trees. The reaction was 1.2% N/100 acid. The sirup contained 95.81% sucrose and 0.34% invert sugar. The color was 3, flavor 2, and score 900.

90. Number 90 was inoculated with *Bacillus acceris*, a stringy sap organism, strain CCXVII. A very deep milky type of souring occurred and the material became quite stringy, though it was by no means as ropy as unsterilized sap inoculated with the same organism. An unpleasant yeasty odor was pro-

nounced. The reaction was 25.4% N/100 acid. The sirup contained 80.72% sucrose and 7.20% invert sugar. The color was 7, flavor 4, and score 600; depreciation from control, color 4, flavor 2, and score 300.

91. Number 91 was inoculated with fluorescent organism CXL. A characteristic green type of souring developed and the organism was recovered. The reaction was 1.5% N/100 acid. The sirup contained 94.76% sucrose and 0.40% invert sugar. The color was 6, flavor 3, and score 750; depreciation from control, color 3, flavor 1 and score 150.

92. Number 92 was inoculated with fluorescent organism CXII. A green type of souring developed and the organism was recovered. The reaction was 1.4% N/100 acid. The sirup contained 95.00% sucrose and 0.52% invert sugar. The color was 7, flavor 3, and score 725; depreciation from control, color 4, flavor 1, and score 175.

93. Number 93 was inoculated with green fluorescent organism LI. Typical green souring developed and the organism was recovered. The reaction was 1.2% N/100 acid. The sirup contained 95.43% sucrose and 0.43 invert sugar. The color was 6, flavor 2, and score 825; depreciation from control, color 3, flavor 0, and score 75.

94. Number 94 was inoculated with green fluorescent organism CXLI. Green souring developed and the organism was recovered. The reaction was 1.2% N/100 acid. The sirup contained 94.68% sucrose and 0.52% invert sugar. The color was 7, flavor 4, and score 600; depreciation from control, color 4, flavor 2, and score 300.

95. Number 95 was inoculated with *Bacillus accris,* a stringy sap organism, strain LXXXVII. A milky type of souring with slight stringiness developed. The organism was recovered. The reaction was 27% N/100 acid. The sirup contained 90.85% sucrose, and 5.04% invert sugar. The color was 6, flavor 3, and score 750; depreciation from control, color 3, flavor 1, and score 150.

96. Number 96 was the control sterilized and placed in the incubator without inoculation. The plates were sterile. The reaction was 0.5% N/100 acid. The sirup contained 95.33% sucrose and 0.44% invert sugar. The color was 5, flavor 2, and score 850; depreciation from control (number 80), color 2, flavor 0, and score 50.

SERIES II: SIRUPS 97 TO 104

The sap of this series was obtained on March 26. It was sterilized at once and again on March 27, and inoculated in the evening of the latter day and incubated for 3½ days. The temperature is shown in the accompanying graph (fig. 12).

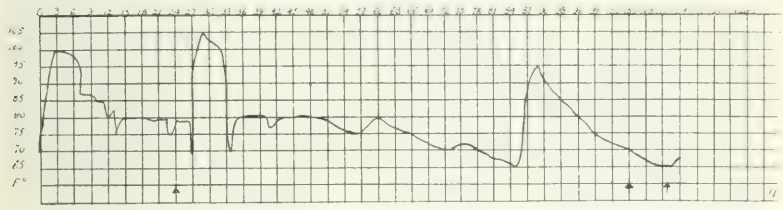

Fig. 12. Graph of incubation temperatures for series II; saps 97 to 104 inclusive. The arrow heads on next to the bottom line from left to right indicate respectively the time of inoculation, the time of evaporation of the first sample and the time of evaporation of the last sample.

97. Number 97 was a control evaporated March 26 immediately after gathering. The reaction was 0.5% N/100 acid. The sirup contained 96.04% sucrose and 0.19% invert sugar. The color was 3, flavor 1, and score 950.

98. Number 98 was inoculated with pink yeast CXXXII. A milky clouding developed accompanied by a very unpleasant odor. The organism was recovered. The reaction was 1.5% N/100 acid. The sirup contained 86.70% sucrose and 8.60% invert sugar. The color was 9, flavor 4, and score 550; depreciation from control, color 6, flavor 3, and score 400.

99. Number 99 was inoculated with red yeast CX. A reddish brown type of souring developed, accompanied by a yeasty odor. The organism was recovered. The reaction was 1.4% N/100 acid. The sirup contained 94.82% sucrose and 2.05%

invert sugar. The color was 7, flavor 4, and score 600; depreciation from control, color 4, flavor 3, and score 350.

100. Number 100 was inoculated with fluorescent organism CXLVIII. A green type of souring developed and the organism was recovered. The reaction was 1.1% N/100 acid. The sirup contained 96.21% sucrose and 0.93% invert sugar. The color was 7, flavor 3, and score 725; depreciation from control, color 4, flavor 2, and score 225.

101. Number 101 was inoculated with fluorescent organism 5. A characteristic type of souring appeared and the organism was recovered. The reaction was 1.2% N/100 acid. The sirup contained 96.45% sucrose and 0.94% invert sugar. The color was 8, flavor 3, and score 700; depreciation from control, color 5, flavor 2, and score 250.

102. Number 102 was inoculated with green fluorescent organism XXXIII. A green type of souring developed and the organism was recovered. The reaction was 4.5% N/100 acid. The sirup contained 95.67% sucrose and 1.14% invert sugar. The color was 7, flavor 4, and score 600; depreciation from control, color 4, flavor 3, and score 350.

103. Number 103 was inoculated with green fluorescent organism XXXVI. A green type of souring developed and the organism was recovered. The reaction was 1.4% N/100 acid. The sirup contained 95.38% sucrose and 1.35% invert sugar. The color was 7, flavor 4, and score 600; depreciation from control, color 4, flavor 3, and score 350.

104. Number 104 was a control sterilized and placed in the incubator without inoculation. The plates were sterile. The reaction was 0.6% N/100 acid. The sirup contained 97.00% sucrose and 0.41% invert sugar. The color was 4, flavor 2, and score 875; depreciation from control (number 97), color 1, flavor 1, and score 75.

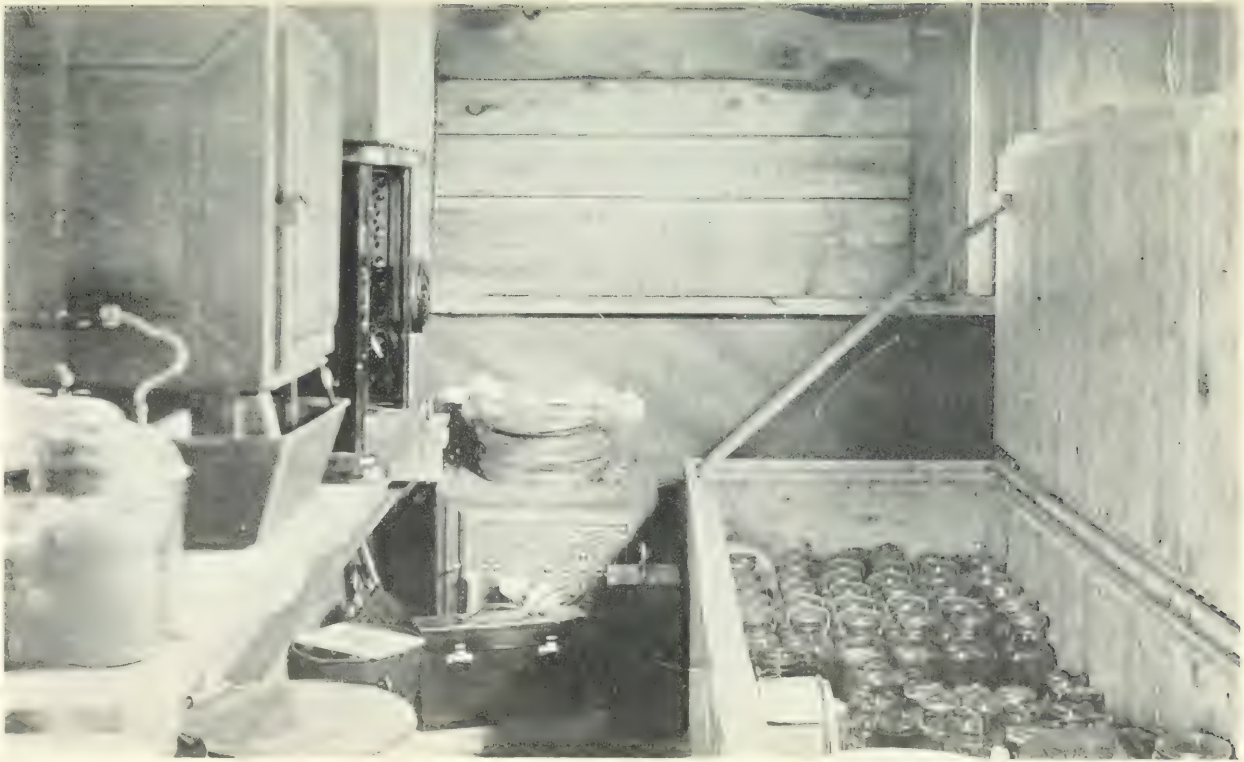

PLATE III. Interior view of the field laboratory. (See page 379).

PLATE IV.—Incubator. (See pages 359-360).

SERIES 12: SIRUPS 105 TO 112

The sap for this series was collected on March 27, given two sterilizations, inoculated on the morning of March 29, and incubated for 3 days at the temperatures indicated in the accompanying graph (figure 13).

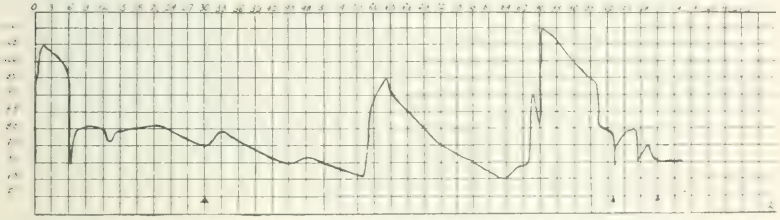

Fig. 13. Graph of incubation temperatures for series 12: saps 105 to 112 inclusive. The arrow heads on next to the bottom line from left to right indicate respectively the time of inoculation, the time of evaporation of the first sample and the time of evaporation of the last sample.

105. Number 105 was a control evaporated on the morning of March 28. The reaction was 1% N/100 acid. The sirup contained 96.23% sucrose and 0.24 invert sugar. The color was 3, flavor 2, and score 900.

106. Number 106 was inoculated with red yeast CCIII, which evidently made a feeble growth as the organism was not recovered from the rather high dilutions employed in plating. The reaction was 4.1% N/100 acid. The sirup contained 97.30% sucrose and 0.66% invert sugar. The color was 7, flavor 3, and score 725; depreciation from control, color 4, flavor 1, and score 175.

107. Number 107 was inoculated with pink coccus CVI. A pale milky type of souring developed. The organism was recovered. The reaction was 4% N/100 acid. The sirup contained 94.52% sucrose and 0.69% invert sugar. The color was 5, flavor 4, and score 650; depreciation from control, color 2, flavor 2, and score 250.

108. Number 108 was inoculated with pink yeast CLXXVIII, but only one colony was recovered. The reaction was 3.3% N/100 acid. The sirup contained 95.37% sucrose and

0.90% invert sugar. The color was 4, flavor 3, and score 800; depreciation from control, color 1, flavor 1, and score 100.

109. Number 109 was inoculated with gray yeast 24. The organism made a remarkable growth as evidenced by the deep clouding of the material and a flaky sediment, as well as by the semi-mycelial growth suspended in the sap. A distinctly yeasty odor developed which became very offensive when the material was heated for evaporation. The specific organism was recovered in great numbers. The reaction was 41% N/100 acid. The sirup contained 67.33% sucrose and 28.25% invert sugar. The color was 10, flavor 4, and score 525; depreciation from control, color 7, flavor 2, and score 375.

110. Number 110 was inoculated with gray yeast CXXI. The organism was recovered from the material which developed a dull brownish white appearance with clouding. The reaction was 3.3% N/100 acid. The sirup contained 97.09% sucrose and 1.74% invert sugar. The color was 6, flavor 4, and score 625; depreciation from control, color 3, flavor 2, and score 275.

111. Number 111 was inoculated with green mold CCCI belonging to the Eurotium genus. The organism made moderate growth as could be seen by the tufts of mycelium developing in the material and also from the plates. The reaction was 2% N/100 acid. The sirup contained 93.31% sucrose and 4.39% invert sugar. The color was 7, flavor 4, and score 600; depreciation from control, flavor 4, color 2, and score 300.

112. Number 112 was the control sterilized and held in the incubator without inoculation. The reaction was 1% N/100 acid. The sirup contained 97.60% sucrose and 1.11% invert sugar. The color was 5, flavor 2, and score 850; depreciation from control (number 105), color 2, flavor 0, and score 50.

SERIES 13: SIRUPS 113 TO 120

The sap for this series flowed on March 30, and was stored outdoors over night at a temperature below freezing, as shown by the ice which formed in the cans during the night. The

material was sterilized on the mornings of March 31, April 1 and April 2, inoculated when cool, and incubated at temperatures indicated in the graph (figure 14).

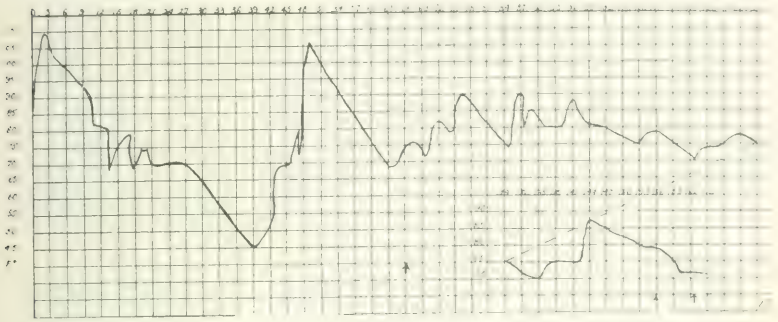

Fig. 14. Graph of incubation temperatures for series B 3; saps 113 to 120 inclusive. The arrow heads on next to the bottom line from left to right indicate respectively the time of inoculation, the time of evaporation of the first sample and the time of evaporation of the last sample.

113. Number 113 was a control evaporated on the morning of March 31. The reaction was 2% N/100 acid. The sirup contained 96.97% sucrose and 0.01% invert sugar. The color was 4, flavor 2, and score 875.

114. Number 114 was inoculated with red yeast LXII. A reddish type of clouding developed and the organism was recovered in large numbers. The reaction was 5% N/100 acid. The sirup contained 96.31% sucrose and 1.01% invert sugar. The color was 5, flavor 3, and score 775; depreciation from control, color 1, flavor 1, and score 100.

115. Number 115 was inoculated with gray yeast CXXI. A milky type of souring developed. The organism was recovered. The reaction was 4.7% N/100 acid. The sirup contained 95.14% sucrose and 1.52% invert sugar. The color was 7, flavor 4, and score 600; depreciation from control, color 3, flavor 2, and score 275.

116. Number 116 was a composite inoculated with red yeast LXII and fluorescent pseudomonas CXLV. A green type of souring developed. The fluorescent organisms appeared in large numbers on the plates, but the yeast was not recovered.

The reaction was 3.7% N/100 acid. The sirup contained 95.62% sucrose and 0.68% invert sugar. The color was 8, flavor 4, and score 575; depreciation from control, color 4, flavor 2, and score 300.

117. Number 117 was inoculated with green mold CCCI belonging to the Eurotium genus. An excellent growth occurred. Some of the tufts of mycelium rose to the top and developed characteristic green spores. The reaction was 7.4% N/100 acid. The sirup contained 91.09% sucrose and 3.72% invert sugar. The color was 9, flavor 4, and score 550; depreciation from control, color 5, flavor 2, and score 325.

118. Number 118 was a composite inoculated with gray yeast CXXI and fluorescent pseudomonas CXLV. The bacteria were recovered in great numbers in association with a few yeast colonies. The type of souring was characteristic of the fluorescent organism, but there was also evidence of the odor which is associated with the development of yeast. The reaction was 4.8% N/100 acid. The sirup contained 95.69% sucrose and 0.98% invert sugar. The color was 7, flavor 4, and score 600; depreciation from control, color 3, flavor 2, and score 275.

119. Number 119 was a composite inoculated with Eurotium CCCI and fluorescent pseudomonas CXLV. The mold apparently made only very slight growth, while the bacteria developed luxuriantly. The reaction was 4.2% N/100 acid. The sirup contained 96.27% sucrose and 0.81% invert sugar. The color was 7, flavor 2, and score 800; depreciation from control, color 3, flavor 0, and score 75.

120. Number 120 was an incubator control, sterilized and held without inoculation. The reaction was 4.6% N/100 acid. The sirup contained 95.02% sucrose and 0.57% invert sugar. The color was 5, flavor 3, and score 775; depreciation from control (number 113), color 1, flavor 1, and score 100.

SIRUPS FROM LAST RUN SAP IN 1911

As in two preceding years, as soon as the season was sufficiently advanced, trees which were running a sour sap were selected to be retapped in order to procure material from both fresh and sour tap-holes of the same tree at the same time.

121. Number 121 was made from sour sap obtained on April 21, after the buds were beginning to show considerable green. The sap was cloudy but not excessively so. The sirup contained 93.94% sucrose and 0.34% invert sugar. The color was 7, flavor 5, and score 450; depreciation from control (number 122), color 2, flavor 0, and score 50; depreciation from the first run control of the season (number 89), color 4, flavor 3, score 450.

122. Number 122 was made from sap obtained from the same tree as 121 and at the same time but from the fresh tap-hole. The sirup contained 95.80% sucrose and 0.07% invert sugar. The color was 5, flavor 5, and score 500; depreciation from first run control, color 2, flavor 3, and score 400.

123. Number 123 was made from sap obtained April 24 from a sour tap-hole. The sirup contained 95.33% sucrose and 0.64% invert sugar. The color was 11, flavor 6, and score 250; depreciation from control (number 124), color 6, flavor 1, and score 250; depreciation from first run control, color 8, flavor 4, and score 650.

124. Number 124 was made from sap obtained from the same tree and at the same time as that for 123, but from a fresh tap-hole. It contained 98.54% sucrose and 0.59% invert sugar. The color was 5, flavor 5, and score 500; depreciation from first run control, color 2, flavor 3, and score 400.

125. Number 125 was made from sour sap obtained from the tree April 24. It contained 93.88% sucrose and 0.53% invert sugar. The color was 7, flavor 5, and score 450; depreciation from control (number 126), color 1, flavor 0, and score 25; depreciation from first run control, color 4, flavor 3, and score 450.

126. Number 126 was obtained from the same tree and at the same time as number 125, but from a fresh tap-hole. The sirup contained 94.46% sucrose and 0.31% invert sugar. The color was 6, flavor 5, and score 475; depreciation from first run control, color 3, flavor 3, and score 425.

127. Number 127 was made from sour sap obtained April 27. The sirup contained 87.41% sucrose and 4.59% invert sugar. The color was 15, flavor 0, and score 150; depreciation from control (number 128), color 11, flavor 1, and score 375; depreciation from first run control, color 12, flavor 4, and score 750.

128. Number 128 was made from sap obtained from the same tree at the same time as that for 127 but from a fresh tap-hole. The sirup contained 94.21% sucrose and 0.31% invert sugar. The color was 4, flavor 5, and score 525; depreciation from first run control, color 1, flavor 3, and score 375.

129. Number 129 was obtained from a fresh tap-hole April 28. It contained 95.34% sucrose and 0.12% invert sugar. The color was 5, flavor 5, and score 500; depreciation from first run control, color 2, flavor 3, and score 400.

130. Number 130 was made from sap obtained from the same tree and at the same time as that for 129, but from the sour tap-hole. It contained 93.80% sucrose and 1.34% invert sugar. The color was 12, flavor 0, and score 225; depreciation from control (number 129), color 7, flavor 1, and score 275; depreciation from first run control, color 0, flavor 4, and score 675.

The results of this series of experiments confirm those obtained the previous year, indicating that, accompanying the resumption of vegetative activity in the tree, there is a change in sap which makes it impossible thereafter to produce sirups of superior flavor.

STATISTICAL SUMMARY OF FIELD EXPERIMENTS

For the sake of convenient reference a summary of the statistical data presented in the preceding pages is appended in tabular form and in numerical order (table 9). The number of the sample, the character of the organism employed or of the sample, the designation of the organism, the bacterial count per cc. of the sap, and the reaction of the sap in percent of N 100 acid occupy the first five columns respectively. These are followed by the percentages of sucrose and invert sugar in the sirups, calculated to a dry matter basis. The numbers in the column headed "ratio" were obtained by dividing the percent of sucrose by the percent of invert sugar. A small ratio number, therefore, indicates a relatively high proportion of invert sugar and a high number, a small proportion. The ratio numbers with their corresponding invert sugar values are shown below in tabular form:

Ratio numbers	Invert sugar values
1 to 5	above 15 %
5 " 10	15 % to 8½%
10 " 20	8½% " 4½%
20 " 50	4½% " 2 %
50 " 90	2 % " 1 %
90 " 180	1 % " ½%
180 " 400	½% " ¼%
above 400	below ¼%

Following the ratio column are given the values for color, flavor and score, assigned to the various sirups, and also the depreciation in color, flavor and score of inoculated sirups from the corresponding value assigned to their respective controls. A date column completes the table.

TABLE 9. TABULAR SUMMARY OF STATISTICAL DATA

No.	Character of sample or organism	Organism number	Count	Reaction	Sucrose
1	Control,				96.84
2	Fluorescent,	XXXIII			96.79
3	Ditto,	XXXVI			96.73
4	Ditto,	L			96.54
5	Ditto,	LIII			97.16
6	Control,				95.41
7	Fluorescent,	LVI			96.16
8	Ditto,	5			97.14
9	Non-fluorescent,	XXVI			95.61
10	Ditto,	XXX			95.86
11	*B. aceris*,	LXXXVII		170.0	96.12
12	Stringy peas,	I			95.71
13	Gray yeast,	24			93.34
14	Red yeast,	25		9.0	95.30
15	Ditto,	LXII		5.0	93.26
16	Penicillium,	LXIV		8.2	95.73
17	Ditto,	LXV		8.0	92.76
18	Red yeast,	LXXIX		12.0	91.65
19	Composite,			5.0	93.28
20	Control,			2.0	95.37
21	Last run, sweet,				95.19
22	Ditto, sour,				95.27
23	Ditto, sour				92.56
24	Ditto, sweet,				96.02
25	Ditto, sour,				94.05
26	Ditto, sweet,				96.42
27	Control,		10000		96.19
28	Gray yeast,	24	437000		82.83
29	Fluorescent,	CXXXIX	12350000		94.91
30	Ditto,	CLIX	3262000		93.75
*31	Gray yeast,	CXXI	5667000		96.62
*32	Ditto,	CLXXX	5600000		95.91
*33	Ditto,	CLXVIII	1950000		95.61
34	Control, 40°		7312000		95.86
35	Red yeast,	CXL	2255000		95.13
*36	Ditto,	CII	7312000		96.16
*37	Ditto,	CLXXV	8825000		95.88
38	Control, 80°		162500		94.80
*39	Gray yeast,	C	9750000		96.18
40	Incubator control,		5850000		95.05
41	Control,		66	1.2	97.82
42	Red yeast,	CVII	13650000	19.0	93.27
43	Control, 100°		1625000	6.5	94.19
44	Fluorescent,	CXLVIII	9000000	10.0	93.03
45	Incubator control,		19500	4.5	96.28
46	Pink coccus,	CIV	14300000	10.0	95.07

*Infection failed.

TABLE 9. TABULAR SUMMARY OF STATISTICAL DATA

					DEPRECIATION			
Inv. Sug.	Ratio	Color	Flavor	Score	Color	Flavor	Score	Date
1.07	90	7	1	850	1	0	25	3-24-09
1.55	62	14	4	425	8	3	450	"
2.09	46	11	2	700	5	1	175	"
1.56	62	9	2	750	3	1	125	"
1.44	67	11	2	700	5	1	175	"
0.97	98	6	1	875				"
1.64	59	10	2	725	4	1	150	3-27-09
1.15	84	10	2	725	4	1	150	"
0.78	122	5	2	850	-1	1	25	"
0.77	124	6	3	750	0	2	125	"
1.72	56	7	4	600	1	3	275	"
2.23	43	6	3	750	0	2	125	"
2.53	37	7	2	800	1	1	75	4- 1-09
3.01	32	10	2	725	4	1	150	"
2.98	31	8	2	775	2	1	100	"
2.61	37	10	4	525	4	3	350	"
3.47	27	10	2	725	4	1	150	"
3.12	29	10	2	725	4	1	150	"
4.09	23	9	3	675	3	2	200	"
1.58	60	6	1	875				"
0.82	116	3	1	950				4-12-09
2.76	35	8	3	700	5	2	250	"
3.55	26	14	3	550	7	2	300	"
0.61	157	7	1	850				"
3.13	30	14	3	550	9	2	350	4-16-09
0.51	189	5	1	900				"
0.30	321	4	2	875				3- 7-10
12.15	7	11	4	500	7	2	375	"
0.38	250	8	2[1]	750	4	0[1]	125	"
0.89	105	8	2	775	4	0	100	"
0.30	322	6	2	825	2	0	50	"
0.46	208	6	2	825	2	0	50	"
0.23	416	7	2	800	3	0	75	"
0.42	228	6	2[1]	800	2	0[1]	75	"
1.15	83	12	2[1]	650	8	0[1]	225	"
0.61	158	9	2[1]	725	5	0[1]	150	"
0.64	150	11	2[1]	675	7	0[1]	200	"
0.48	198	5	2[1]	825	1	0[1]	50	"
0.26	370	7	2	800	3	0	75	"
0.64	149	9	2	750	5	0	125	"
0.45	217	4	1	925				3-14-10
2.14	44	5	2[2]	800	1	1[2]	125	"
2.64	36	7	2	800	3	1	125	"
1.40	67	9	3	675	5	2	250	"
0.51	189	4	1	925	0	0	0	"
1.05	91	6	2	825	2	1	100	"

TABLE 9—*Continued*

No.	Character of sample or organism	Organism number	Count	Reaction	Sucrose
47	Pink coccus,	IV	1950000	9.6	88.81
48	Gray yeast,	CLXXX	1625000	16.7	93.89
49	Control,		100	1.0	96.51
50	Pink coccus,	XLIX	1950000	8.0	93.83
51	Ditto,	CVI	4225000	3.5	97.30
52	Gray yeast,	CLXII	1275000	6.0	96.84
53	Ditto,	CLXXI	630000	6.0	92.13
54	Pink coccus,	CXXVI	1300000	8.5	94.11
55	Non-fluorescent,	CVIII	2730000	6.0	94.76
56	Control, 100		320000	3.1	96.02
57	Incubator control.		37000	2.5	96.47
58	Control,		380	3.0	97.01
59	Red yeast.	CXXIII	325000	8.0	94.74
60	Ditto,	CXIII	1950000	8.4	95.44
61	Ditto,	CX	650000	5.5	95.80
62	Ditto,	25	650000	9.5	89.27
63	Ditto,	CXIV	975000	11.0	89.06
64	Ditto,	CXX	97500	10.0	91.64
65	Incubator control,		1950000	9.0	91.89
66	Tin buckets,		140	2.0	96.62
67	Wood buckets,		6500000	5.5	88.25
68	Control		500	2.5	96.90
69	Fluorescent,	CLIV	19500000	10.0	93.87
70	Ditto,	CXXXIII	20400000	10.0	93.47
71	Ditto,	CXLVII	23400000	6.5	93.69
72	Ditto,	CLIII	29250000	10.0	95.07
73	Ditto,	CLVIII	23400000	2.3	97.47
*74	Control burned.		750	3.0	96.36
*75	Fluorescent,	CLXXIX	10400000	10.0	84.74
*76	Ditto,	CLV	4875000	10.7	87.03
*77	Ditto,	CLXXVII	11700000	10.1	88.13
*78	Ditto,	XXXVI	14625000	11.2	83.86
*79	Non-fluorescent,	CLXIII	6500000	25.4	78.78
*80	Pink yeast,	CLXXVIII	9750000	12.5	79.20
*81	Incubator control,		3250000	10.0	89.23
†82	Sour sap,				
†83	Ditto,		43875000	6.0	
84	Sour sap kept,				91.14
85	Ditto,		11275000	6.0	94.68
86	Ditto,		11275000	6.0	93.21
87	Last run, fresh,		5	2.5	97.27
88	Ditto, sour,		73125000	4.0	94.56
89	Control,			1.2	95.81
90	*B. aceris.*	CCXVII		25.4	86.72
91	Fluorescent.	CXL		1.5	94.76

*A spore bearing organism with inverting power was present in association with the introduced species.

†82 and 83, made into sugar.

TABLE 9.—*Continued*

					DEPRECIATION			
Inv. Sug.	Ratio	Color	Flavor	Score	Color	Flavor	Score	Date
7.90	11	6	3	750	2	2	175	3-14-10
2.35	40	6	2	775	2	1	150	"
0.42	230	2	1	975				3-20-10
2.20	43	6	2	800	4	1	175	"
0.65	150	4	2	875	2	1	100	"
1.21	80	5	2	850	3	1	125	"
3.96	23	6	3	750	4	2	225	"
2.71	35	7	2	750	5	1	225	"
2.38	40	5	2	850	3	1	125	"
1.35	71	5	2	850	3	1	125	"
0.60	161	2	1	975	0	0	0	"
0.43	226	3	1	950				3-21-10
2.03	47	7	3	725	4	2	225	"
1.76	54	6	3	750	3	2	200	"
1.77	54	9	3	675	6	2	275	"
7.30	12	9	4	550	6	3	400	"
7.63	12	8	3	700	5	2	250	"
5.86	16	8	3	700	5	2	250	"
3.52	26	6	4	625	3	3	325	"
0.41	236	3	1	950				3-22-10
6.91	13	9	4	550	6	3	400	3-24-10
0.51	190	4	1	925				3-22-10
1.59	59	9	3	675	5	2	250	"
2.46	38	11	4	500	7	3	425	"
0.58	162	14	4	425	10	3	500	"
0.87	109	13	4	450	9	3	475	"
0.68	143	6	2	825	2	1	100	"
0.70	138	9	4	550	5	3	375	3-24-10
10.77	8	9	3	675	5	2	250	"
8.94	10	10	3	650	6	2	275	"
8.24	11	8	3	700	4	2	225	"
10.48	8	8	4	575	4	3	350	"
15.25	5	7	4	600	3	3	325	"
16.13	5	10	4	525	6	3	400	"
7.51	12	7	4	600	3	3	325	"
								3-29-10
								4- 2-10
2.83	32	20	3*	350				"
1.57	60	20*	3*	325				"
1.23	76	20*	3*	325				"
0.26	374	4	5	525				4- 3-10
1.79	53	11	6	250	7	1	275	"
0.34	282	3	2	900				3-22-11
7.20	12	7	4	600	4	2	300	"
0.40	237	6	3	750	3	1	150	"

*A spore bearing organism with inverting power was present in association with the introduced species.

TABLE 9—*Concluded*

No.	Character of sample or organism	Organism number	Count	Reaction	Sucrose
92	Fluorescent,	CXII		1.4	95.00
93	Ditto,	LI		1.2	95.43
94	Ditto,	CXLI		1.2	94.68
95	*B. aceris*,	LXXXVII		27.0	90.85
96	Incubator control,			0.5	95.33
97	Control,			0.5	96.04
98	Pink yeast,	CXXXII		1.5	86.70
99	Red yeast,	CX		1.4	94.82
100	Fluorescent,	CXLVIII		1.1	96.21
101	Ditto,	5		1.2	96.45
102	Ditto,	XXXIII		4.5	95.67
103	Ditto,	XXXVI		1.4	95.38
104	Incubator control,			0.6	97.00
105	Control,			1.0	96.23
‡106	Red yeast,	CCIII		4.1	97.30
107	Pink coccus,	CVI		4.0	94.52
‡108	Pink yeast,	CLXXVIII		3.3	95.37
109	Gray yeast,	24		41.0	67.33
110	Ditto,	CXXI		3.3	97.09
111	Eurotium,	CCCI		2.0	93.31
112	Incubator control,			1.0	97.60
113	Control,			2.0	96.97
114	Red yeast,	LXII		5.0	96.31
115	Gray yeast,	CXXI		4.7	95.14
116	Composite,	LXII CXLV		3.7	95.62
117	Eurotium,	CCCI		7.4	91.09
118	Composite,	CXXI CXLV		4.8	95.69
119	Composite,	CCCI CXLV		4.2	96.27
120	Incubator control,			6.4	95.02
121	Last run sour				93.94
122	Ditto, sweet				95.80
123	Ditto, sour				95.33
124	Ditto, sweet				98.54
125	Ditto, sour				93.88
126	Ditto, sweet				94.46
127	Ditto, sour				87.41
128	Ditto, sweet				94.21
129	Ditto, sweet				95.34
130	Ditto, sour				93.86

‡106 and 108, infection failed.

TABLE 9—*Concluded*

					DEPRECIATION			
Inv. Sug.	Ratio	Color	Flavor	Score	Color	Flavor	Score	Date
0.52	183	7	3	725	4	1	175	3-22-11
0.43	222	6	2	825	3	0	75	"
0.52	182	7	4	600	4	2	300	"
5.04	18	6	3	750	3	1	150	"
0.44	217	5	2	850	2	0	50	"
0.19	505	3	1	950				3-26-11
8.69	10	9	4	550	6	3	400	"
2.05	46	7	4	600	4	3	350	"
0.93	103	7	3	725	4	2	225	"
0.94	103	8	3	700	5	2	250	"
1.14	84	7	4	600	4	3	350	"
1.35	71	7	4	600	4	3	350	"
0.41	237	4	2	875	1	1	75	"
0.24	403	3	2	900				3-27-11
0.66	147	7	3	725	4	1	175	"
0.69	137	5	4	650	2	2	250	"
0.90	106	4	3	800	1	1	100	"
28.35	2	10	4	525	7	2	375	"
1.74	56	6	4	625	3	2	275	"
4.39	21	7	4	600	4	2	300	"
1.11	88	5	2	850	2	0	50	"
0.61	159	4	2	875				3-30-11
1.01	95	5	3	775	1	1	100	"
1.52	63	7	4	600	3	2	275	"
0.68	141	8	4	575	4	2	300	"
3.72	25	9	4	550	5	2	325	"
0.98	98	7	4	600	3	2	275	" *
0.81	119	7	2	800	3	0	75	"
0.57	167	5	3	775	1	1	100	" *
0.34	276	7	5	450	2	0	50	4-21-11
0.67	143	5	5	500				"
0.64	149	11	6	250	6	1	250	4-24-11
0.59	167	5	5	500				"
0.53	125	7	5	450	1	0	25	"
0.31	305	6	5	475				"
4.59	19	15	6	150	11	1	375	4-27-11
0.31	304	4	5	525				"
0.12	794	5	5	500				4-28-11
1.34	70	12	6	225	7	1	275	"

DISCUSSION OF RELATED SIRUPS IN GROUPS

A survey of the preceding table shows that the sirups naturally fall into different groups according to the character of the sap used or of the treatment accorded to it. For convenient study and discussion the tables have been re-arranged so as to bring similar samples together in groups.

It appeared in the discussion of individual samples that the inoculation failed in certain instances. Such samples have been placed by themselves and are grouped together without regard to the character of the organisms with which inoculation was attempted. In one series a spore-bearing organism became at least equally as important as the bacteria which were artificially introduced, and this series has been treated as a unit in the re-arrangement. The samples inoculated with yeasts and molds showed a tendency to mixed infection in which the fluorescent organisms naturally present played a more or less important part. With the other statistical data in the following tables, the average color, flavor and score for each group, as well as the average depreciation of color, flavor and score, is recorded at the foot in the proper columns.

Twenty-two sirups were made from sap successfully inoculated with one or another strain of fluorescent bacteria. The average color was 9, flavor 2.0, and score 665; the average depreciation from control, color 4.8, flavor 1.6, and score 242.

Four samples were successfully inoculated with non-fluorescent bacteria. The average color was 5.5, flavor 2.5, and score 800; the average depreciation from control, color 0.5, flavor 2.5, and score 100.

Seven samples were influenced by the action of a spore-bearing organism of the subtilis type which appeared spontaneously. Four of these were inoculated with fluorescent organisms, one with a non-fluorescent bacillus similar to the intruder, and one with a pink yeast which was not recovered, while the other was intended for an incubator control. It is evident that the quality of these sirups must be attributed to the combined action of the two groups of organisms. The average color was

8.4, flavor 3.0, and score 618; the average depreciation from control, color 4.4, flavor 2.0, and score 307.

Three sirups were made from sap successfully inoculated with *Bacillus aceris*, a stringy sap organism, of which two strains were employed. The average color was 0.7, flavor 3.7 and score 650; the average depreciation from control, color 2.7, flavor 2, and score 242.

Six samples were inoculated with pink cocci belonging to the *Micrococcus roseus* type. The average color was 5.7, flavor 2.0, and score 775; the average depreciation from control, color 2.8, flavor 1.6, and score 171.

Only one sample was successfully inoculated with pink yeast.

Thirteen samples were inoculated with red yeast with at least partial success. The fluorescent bacteria developed in association with the introduced organisms in a considerable number of these samples, so that the quality of sirup secured must be assigned to the combined activities of the two species. The average color was 8, flavor 2.8, and score 703; the average depreciation from control, color 4.1, flavor 1.7, and score 215.

Eight samples were inoculated with gray yeast with partial or complete success. Here again the fluorescent bacteria sometimes developed in association with the yeasts and the results must be regarded as the product of the activities of both classes of organisms. The average color was 7.3, flavor 3.2, and score 678; the average depreciation from control, color 3.8, flavor 1.7, and score 234.

Four samples were successfully inoculated with green mold. In two instances Penicillium was employed and in the others, Eurotium. Fluorescent bacteria played a part as associated organisms in certain of these samples. The average color was 9, flavor 3.5, and score 600; average depreciation from control, color 4.8, flavor 2.0, and score 281.

Four sirups were made from saps inoculated with a mixture of organisms. The composite infection was undertaken to determine the influence of yeasts and molds upon the development of fluorescent bacteria. The results were not entirely satisfactory

but indicate that when these two groups of organisms are associated in the same sample of sap the fluorescent bacteria are likely to gain the ascendency. The appearance of the samples and the results of the plates indicated that the development of the fluorescent group was stimulated by the presence of yeasts and molds, with which they must compete. This may account for the considerable number of failures which resulted from attempts to inoculate unsterilized sap with cultures of yeasts and molds. The average figures for the composite samples follow: Color 7.7, flavor 3, and score 663; the average depreciation from control, color 3.3, flavor 1.5, and score 213.

In eight cases, attempts at infection failed or at least the introduced organism was not recovered. In a majority of these samples the fluorescent bacteria appeared sooner or later and exercised an influence upon the quality of the sirup. The average color was 7, flavor 2.3, and score 772; average depreciation from control, color 3.4, flavor 0.3, and score 103.

Three sirups were made from natural sour sap, that is to say, sap which was allowed to remain in the buckets late in the season until it had seriously depreciated. One object of this procedure was to determine what proportion of invert sugar might be expected in such material. Difficulty is often experienced in graining sugar made from the last run sirups. This trouble probably results from a large proportion of invert sugar, which of course might be formed in the sap by the action of micro-organisms. In the three sirups here reported, however, the proportion of invert sugar was low. Two other samples of similar material yielded sugar which grained readily. The average figures for the three sirups follow: color 20+, flavor 3^1, and score 333; average depreciation calculated on first run control, color 16+, flavor 1^1, and score 542.

Thirteen samples were reserved for incubator controls. These were treated in different ways in different series. Some of them were so handled that they were only slightly inferior to the true control, while in other instances they were very nearly comparable to inoculated material. The greater part of the depreciation in

these samples should be attributed to the fluorescent group of bacteria. The average color was 5.4, flavor 2, and score 827; average depreciation, color 1.8, flavor 0.7, and score 87.

Eleven samples were reserved as controls. Part of these were held in the cold during the incubation period and undoubtedly underwent slight depreciation. The average color was 3.8, flavor 1.4, and score 911.

Nine samples were made from last run sap obtained from tap-holes which had become sour, that is from which cloudy sap was running. The average color was 11, flavor 4.8, and score 397; average depreciation from the first run control of the same season, color 6.9, flavor 3.1, and score 490.

Nine samples were made from last run material obtained from the same trees and at the same time as the nine samples mentioned in the preceding paragraph, but from fresh tap-holes. It is important to notice that the first three samples of this character which were made in 1909 possessed a flavor equal to that of the first run control, and that two of these same sirups were superior in color to their control. In the remaining six instances, however, the product of the two succeeding seasons, the sirups possessed the characteristic buddy flavor and were uniformly 3 grades inferior to the controls. The average color was 5, flavor 3.7, and score 636; average depreciation from first run control of the same season, color 0.8, flavor 2, and score 243.

If the nine samples of sirups obtained from the sour sap of the last run are compared with the nine samples obtained from the sweet sap of the last run, it is seen that the average depreciation from souring is 6.1 in color, 1.1 in flavor, and 239 in score.

One sirup was made from sap caught in tin buckets under cleanly conditions, and one was made from sap obtained the following days from the same tree but caught in wooden buckets. This latter sample showed a depreciation of 6 points in color, 3 in flavor, and 400 in score.

In addition to the groups mentioned above one sirup originally intended for a control was burned so that it was necessary to eliminate it from the comparison. The tabulated results follow:

TABLE 10. STATISTICAL TABLES SHOWING RELATED SAMPLES IN JUXTAPOSITION

No.	Character of sample or organism	Organism number	Count	Reaction	Sucrose
2	Fluorescent,	XXXIII			96.79
102	Ditto,	Ditto,		4.5	95.67
3	Ditto,	XXXVI			96.73
103	Ditto,	Ditto,		1.4	95.38
4	Ditto,	L			96.54
5	Ditto,	LIII			97.16
7	Ditto,	LVI			96.16
8	Ditto,	5			97.14
101	Ditto,	5		1.2	96.45
29	Ditto,	CXXXIX	12350000		94.91
30	Ditto,	CLIX	3262000		93.75
44	Ditto,	CLXVIII	90000000	10.0	93.03
100	Ditto,	Ditto,		1.1	96.21
69	Ditto,	CLIV	19500000	10.0	93.87
70	Ditto,	CXXXIII	20400000	10.0	93.47
71	Ditto,	CXLVII	23400000	6.5	93.69
72	Ditto,	CLIII	29250000	10.0	95.07
73	Ditto,	CLVIII	23400000	2.3	97.47
91	Ditto,	CXL		1.5	94.76
92	Ditto,	CXII		1.4	95.00
93	Ditto,	LI		1.2	95.43
94	Ditto,	CXLI		1.2	94.68
	Average,				
9	Non-fluorescent,	XXVI			95.61
10	Ditto,	XXX			95.86
12	Ditto,	I			95.71
55	Ditto,	CVIII	2730000	6.0	94.76
	Average,				
75	Spore-bearer plus*	CLXXIX	10400000	10.0	84.74
76	Ditto,	CLV	4875000	10.7	87.03
77	Ditto,	CLXXVII	11700000	10.1	88.13
78	Ditto,	XXXVI	14625000	11.2	83.86
79	Ditto,	CLXIII	6500000	25.4	78.78
80	Ditto,	CLXXVIII	9750000	12.5	79.20
81	Ditto,		3250000	10.0	89.23
	Average,				
11	*B. aceris*,	LXXXVII		170.0	96.12
95	Ditto,	Ditto,		27.0	90.85
90	Ditto,	CCXVII		25.4	86.72
	Average,				

*I. e., spore-bearing organisms associated with fluorescent and other organisms. (See pages 375 to 377.)

TABLE 10. STATISTICAL TABLES SHOWING RELATED SAMPLES IN JUXTAPOSITION

					DEPRECIATION			
Inv. Sug.	Ratio	Color	Flavor	Score	Color	Flavor	Score	Date
1.55	62	14	4	425	8	3	450	3-24-09
1.14	84	7	4	600	4	3	350	3-26-11
2.09	46	11	2	700	5	1	175	3-24-09
1.35	71	7	4	600	4	3	350	3-26-11
1.56	62	9	2	750	3	1	125	3-24-09
1.44	67	11	2	700	5	1	175	"
1.64	59	10	2	725	4	1	150	3-27-09
1.15	84	10	2	725	4	1	150	"
0.94	103	8	3	700	5	2	250	3-26-11
0.38	250	8	2[1]	750	4	0[1]	125	3- 7-10
0.89	105	8	2	775	4	0	100	"
1.40	67	9	3	675	5	2	250	3-14-10
0.93	103	7	3	725	4	2	225	3-26-11
1.59	59	9	3	675	5	2	250	3-22-10
2.46	38	11	4	500	7	3	425	"
0.58	162	14	4	425	10	3	500	"
0.87	109	13	4	450	9	3	475	"
0.68	143	6	2	825	2	1	100	"
0.40	237	6	3	750	3	1	150	3-22-11
0.52	183	7	3	725	4	1	175	"
0.43	222	6	2	825	3	0	75	"
0.52	182	7	4	600	4	2	300	"
		9	2.9	665	4.8	1.6	242	
0.78	122	5	2	850	—1	1	25	3-27-09
0.77	124	6	3	750	0	2	125	"
2.23	43	6	3	750	0	2	125	"
2.38	40	5	2	850	3	1	125	3-20-10
		5.5	2.5	800	0.5	1.5	100	
10.77	8	9	3	675	5	2	250	3-24-10
8.94	10	10	3	650	6	2	275	"
8.24	11	8	3	700	4	2	225	"
10.45	8	8	4	575	4	3	350	"
15.25	5	7	4	600	3	3	325	"
16.13	5	10	4	525	6	3	400	"
7.51	12	7	4	600	3	3	325	"
		8.4	3.6	618	4.4	2.6	307	
1.72	56	7	4	600	1	3	275	3-27-09
5.04	18	6	3	750	3	1	150	3-22-11
7.20	12	7	4	600	4	2	300	"
		6.7	3.7	650	2.7	2	242	

TABLE 10--*Continued*

No.	Character of sample or organism	Organism number	Count	Reaction	Sucrose
46	Pink coccus,	CIV	14300000	10.0	95.07
47	Ditto,	IV	1950000	9.6	88.81
50	Ditto,	XLIX	1950000	8.0	93.83
51	Ditto,	CVI	4225000	3.5	97.30
107	Ditto,	Ditto,		4.0	94.52
54	Ditto,	CXXVI	1300000	8.5	94.11
	Average,				
98	Pink yeast,	CXXXII		1.5	86.70
14	Red yeast,	25		9.0	95.30
62	Ditto,	Ditto,	650000	9.5	89.27
15	Ditto,	LXII		5.0	93.26
114	Ditto,	Ditto,		5.0	96.31
18	Ditto,	LXXIX		12.0	91.65
35	Ditto,	CXI	2255000		95.13
42	Ditto,	CVII	13650000	19.0	93.27
59	Ditto,	CXXIII	325000	8.0	94.74
60	Ditto,	CXIII	1950000	8.4	95.44
61	Ditto,	CX	650000	5.5	95.80
99	Ditto,	Ditto,		1.4	94.82
63	Ditto,	CXIV	975000	11.0	89.06
64	Ditto,	CXX	97500	10.0	91.64
	Average,				
13	Gray yeast,	24			93.34
28	Ditto,	Ditto,	437000		82.83
109	Ditto,	Ditto.		41.0	67.33
48	Ditto,	CLXXX	1625000	16.7	93.89
52	Ditto,	CLXII	1275000	6.0	96.84
53	Ditto,	CLXXI	630000	6.0	92.13
110	Ditto,	CXXI		3.3	97.09
115	Ditto,	Ditto		4.7	95.14
	Average,				
16	Green mold,	LXIV		8.2	95.73
17	Ditto,	LXV		8.0	92.76
111	Ditto,	CCCI		2.0	93.31
117	Ditto,	Ditto,		7.4	91.09
	Average,				
19	Composite,			5.0	93.28
116	Ditto,			3.7	95.62
118	Ditto,			4.8	95.69
119	Ditto,			4.2	96.27
	Average,				

TABLE 10—*Continued*

					DEPRECIATION			
Inv. Sug.	Ratio	Color	Flavor	Score	Color	Flavor	Score	Date
1.05	91	6	2	825	2	1	100	3-14-10
7.90	11	6	3	750	2	2	175	"
2.20	43	6	2^1	800	4	1^1	175	3-20-10
0.65	150	4	2	875	2	1	100	"
0.69	137	5	4	650	2	2	250	3-27-11
2.71	35	7	2^2	750	5	1^2	225	3-20-10
		5.7	2.6	775	2.8	1.6	171	
8.69	10	9	4	550	6	3	400	3-26-11
3.01	32	10	2	725	4	1	150	4- 1-09
7.30	12	9	4	550	6	3	400	3-21-10
2.98	31	8	2	775	2	1	100	4- 1-09
1.01	95	5	3	775	1	1	100	3-30-11
3.12	29	10	2	725	4	1	150	4- 1-09
1.15	83	12	2^1	650	8	0^1	225	3- 7-10
2.14	44	5	2^2	800	1	1^2	125	3-14-10
2.03	47	7	3	725	4	2	225	3-21-10
1.76	54	6	3	750	3	2	200	"
1.77	54	9	3	675	6	2	275	"
2.05	46	7	4	600	4	3	350	3-26-11
7.63	12	8	3	700	5	2	250	3-21-10
5.86	16	8	3	700	5	2	250	"
		8	2.8	703	4.1	1.7	215	
2.53	37	7	2	800	1	1	75	4- 1-09
12.15	7	11	4	500	7	2	375	3- 7-10
28.35	2	10	4	525	7	2	375	3-27-11
2.35	40	6	2^2	775	2	1^2	150	3-14-10
1.21	80	5	2	850	3	1	125	3-20-10
3.96	23	6	3	750	4	2	225	"
1.74	56	6	4	625	3	2	275	3-27-11
1.52	63	7	4	600	3	2	275	3-30-11
		7.3	3.2	678	3.8	1.7	234	
2.61	37	10	4	525	4	3	350	4- 1-09
3.47	27	10	2	725	4	1	150	"
4.39	21	7	4	600	4	2	300	3-27-11
3.72	25	9	4	550	5	2	325	3-30-11
		9	3.5	600	4.3	2	281	
4.09	23	9	3	675	3	2	200	4- 1-09
0.68	141	8	4	575	4	2	300	3-30-11
0.98	98	7	4	600	3	2	275	"
0.81	119	7	2	800	3	0	75	"
		7.7	3	663	3.3	1.5	213	

TABLE 10—*Continued*

No.	Character of sample or organism	Organism number	Count	Reaction	Sucrose
31	Failure,		5667000		96.62
32	Ditto,		5600000		95.91
33	Ditto,		1950000		95.61
36	Ditto,		7312000		96.16
37	Ditto,		8825000		95.88
39	Ditto,		9750000		96.18
106	Ditto,			4.1	97.30
108	Ditto,			3.3	95.37
	Average,				
84	Sour sap kept,				91.14
85	Ditto,		11275000	6.0	94.68
86	Ditto,		11275000	6.0	93.21
	Average,				
	Depreciation on foregoing calculated on first run control.				
1	Incubator control,				96.84
34	Ditto,		7312000		95.86
38	Ditto,		162500		94.80
40	Ditto,		5850000		95.05
43	Ditto,		1625000	6.5	94.19
45	Ditto,		19500	4.5	96.28
56	Ditto,		320000	3.1	96.02
57	Ditto,		37000	2.5	96.47
65	Ditto,		1950000	9.0	91.89
96	Ditto,			0.5	95.33
104	Ditto,			0.6	97.00
112	Ditto,			1.0	97.60
120	Ditto,			6.4	95.02
	Average,				
6	Control,				95.41
20	Ditto,			2.0	95.37
27	Ditto,		10000		96.19
41	Ditto,		66	1.2	97.82
49	Ditto,		100	1.0	96.51
58	Ditto,		380	3.0	97.01
68	Ditto,		500	2.5	96.90
89	Ditto,			1.2	95.81
97	Ditto,			0.5	96.04
105	Ditto,			1.0	96.23
113	Ditto,			2.0	96.97
	Average,				

TABLE 10—*Continued*

					DEPRECIATION			
Inv. Sug.	Ratio	Color	Flavor	Score	Color	Flavor	Score	Date
0.30	322	6	2	825	2	0	50	3- 7-10
0.46	208	6	2	825	2	0	50	"
0.23	416	7	2	800	3	0	75	"
0.61	158	9	2[1]	725	5	0[1]	150	"
0.64	150	11	2[1]	675	7	0[1]	200	"
0.26	370	7	2	800	3	0	75	"
0.66	147	7	3	725	4	1	175	3-27-11
0.90	106	4	3	800	1	1	100	"
		7	2.3	772	3.4	.3	103	
2.83	32	20	3[1]	350	16	1[1]	525	4- 2-10
1.57	60	20+	3[1]	325	16+	1[1]	550	"
1.23	76	20+	3[1]	325	16+	1[1]	550	"
		20	3[1]	333	16	1[1]	542	
1.07	90	7	1	850	1	0	25	3-24-09
0.42	228	6	2[1]	800	2	0[1]	75	3- 7-10
0.48	198	5	2[1]	825	1	0[1]	50	"
0.64	149	9	2	750	5	0	125	"
2.64	36	7	2	800	3	1	125	3-14-10
0.51	189	4	1	925	0	0	0	"
1.35	71	5	2	850	3	1	125	3-20-10
0.60	161	2	1	975	0	0	0	"
3.52	26	6	4	625	3	3	325	3-21-10
0.44	217	5	2	850	2	0	50	3-22-11
0.41	237	4	2	875	1	1	75	3-26-11
1.11	88	5	2	850	2	0	50	3-27-11
0.57	167	5	3	775	1	1	100	3-30-11
		5.4	2	827	1.8	.7	87	
0.97	98	6	1	875				3-24-09
1.58	60	6	1	875				4- 1-09
0.30	321	4	2	875				3- 7-10
0.45	217	4	1	925				3-14-10
0.42	230	2	1	975				3-20-10
0.43	226	3	1	950				3-21-10
0.51	190	4	1	925				3-22-10
0.34	282	3	2	900				3-22-11
0.19	505	3	1	950				3-26-11
0.24	403	3	2	900				3-27-11
0.61	159	4	2	875				3-30-11
		3.8	1.4	911				

TABLE 10—*Concluded*

No.	Character of sample or organism	Organism number	Count	Reaction	Sucrose
22	Last run sour,				95.27
23	Ditto,				92.56
25	Ditto,				95.05
88	Ditto,		73125000	4.0	94.56
121	Ditto,				93.94
123	Ditto,				95.33
125	Ditto,				93.88
127	Ditto,				87.41
130	Ditto,				93.86
	Average,				
	Depreciation on foregoing calculated on first run control.				
21	Last run sweet,				95.19
24	Ditto,				96.02
26	Ditto,				96.42
87	Ditto,		5	2.5	97.27
122	Ditto,				95.80
124	Ditto,				98.54
126	Ditto,				94.46
128	Ditto,				94.21
129	Ditto,				95.34
	Average,				
	Depreciation on foregoing calculated on first run control.				
22	Last run sour,				95.27
21	Ditto, sweet,				95.19
23	Ditto, sour,				92.56
24	Ditto, sweet,				96.02
25	Ditto, sour,				94.05
26	Ditto, sweet,				96.42
88	Ditto, sour,		73125000	4.0	94.56
87	Ditto, sweet,		5	2.5	97.27
121	Ditto, sour,				93.94
122	Ditto, sweet,				95.80
123	Ditto, sour,				95.33
124	Ditto, sweet,				98.54
125	Ditto, sour,				93.88
126	Ditto, sweet,				94.46
127	Ditto, sour,				87.41
128	Ditto, sweet,				94.21
130	Ditto, sour,				93.86
129	Ditto, sweet,				95.34
	Average depreciation of sour samples,				
66	Tin bucket,		140	2.0	96.62
67	Wood bucket,		6500000	5.5	88.25
74	Burned control,		750	3.0	96.36

TABLE 10—*Concluded*

					DEPRECIATION			
Inv. Sug.	Ratio	Color	Flavor	Score	Color	Flavor	Score	Date
2.76	35	8	3	700	2	2	175	4-12-09
3.55	26	14	3	550	8	2	325	"
3.13	30	14	3	550	8	2	325	4-16-09
1.79	53	11	6	250	7	4	625	4- 3-10
0.34	276	7	5	450	4	3	450	4-21-11
0.64	149	11	6	250	8	4	650	4-24-11
0.53	125	7	5	450	4	3	450	"
4.59	19	15	6	150	12	4	750	4-27-11
1.34	70	12	6	225	9	4	675	4-28-11
		11	4.8	397	6.9	3.1	492	
0.82	116	3	1	950	-3	0	-75	4-12-09
0.61	157	7	1	850	1	0	25	"
0.51	189	5	1	900	-1	0	-25	4-16-09
0.26	374	4	5	525	0	3	250	4- 3-10
0.67	143	5	5	500	2	3	400	4-21-11
0.59	167	5	5	500	2	3	400	4-24-11
0.31	305	6	5	475	3	3	425	"
0.31	304	4	5	525	1	3	375	4-27-11
0.12	794	5	5	500	2	3	400	4-28-11
		5	3.7	636	.8	2	243	
2.76	35	8	3	700	5	2	250	4-12-09
0.82	116	3	1	950				"
3.55	26	14	3	550	7	2	300	"
0.61	157	7	1	850				"
3.13	30	14	3	550	9	2	350	4-16-09
0.51	189	5	1	900				"
1.79	53	11	6	250	7	1	275	4- 3-10
0.62	374	4	5	525				"
0.34	276	7	5	450	2	0	50	4-21-11
0.67	143	5	5	500				"
0.64	149	11	6	250	6	1	250	4-24-11
0.59	167	5	5	500				"
0.53	125	7	5	450	1	0	25	"
0.31	305	6	5	475				"
4.59	19	15	6	150	11	1	375	4-27-11
0.31	304	4	5	525				"
1.34	794	12	6	225	7	1	275	4-28-11
0.12	70	5	5	500				"
					6.1	1.1	239	
0.41	236	3	1	950				3-22-10
6.91	13	9	4	550	6	3	400	3-24-10
0.70	138	9	4	550	5	3	375	3-24-10

TABLE 11. SUMMARY OF AVERAGES OF RELATED GROUPS IN ORDER OF SCORE

No.	Group	Number of samples in group	Sucrose
1	Tin buckets,	1	96.62
2	Controls,	11	96.32
3	Incubator controls,	13	95.55
4	Non-fluorescent bacteria,	4	95.50
5	Pink cocci,	6	93.94
6	Failures,	8	96.13
7	Red yeasts,	13	93.54
8	Gray yeasts,	8	89.72
9	Fluorescent bacteria,	22	95.42
10	Composites,	4	95.22
11	*B. aceris*,	3	91.16
12	Last run sweet,	9	95.92
13	Fluorescent and spore-bearing bacteria,	7	84.42
14	Green molds,	4	93.21
15	Pink yeast,	1	86.70
16	Wooden buckets	1	88.25
17	Burned control,	1	96.36
18	Last run, sour,	9	93.41
19	Sour sap kept,	3	93.00

TABLE 11. SUMMARY OF AVERAGES OF RELATED GROUPS IN ORDER OF SCORE

Inv. Sug.	Ratio	Color	Flavor	Score	DEPRECIATION Color	Flavor	Score
0.41	236	3.0	1.0	950			
0.60	161	3.8	1.4	911			
1.04	90	5.4	2.0	827	1.8	0.7	87
1.52	62	5.5	2.5	800	0.5	1.5	100
2.45	37	5.7	2.6	775	2.8	1.6	171
0.51	74	7.0	2.3	772	3.4	0.3	103
3.18	29	8.0	2.8	703	4.1	1.7	215
6.86	13	7.3	3.2	678	3.8	1.7	234
1.09	88	9.0	2.9	665	4.8	1.6	242
1.64	58	7.7	3.0	663	3.3	1.5	213
4.70	20	6.7	3.7	650	2.7	2.0	242
0.47	204	5.0	3.7	636	0.8	2.0	243
11.03	8	8.4	3.6	618	4.4	2.6	307
3.56	26	9.0	3.5	600	4.3	2.0	281
8.69	10	9.0	4.0	550	6.0	3.0	400
6.91	13	9.0	4.0	550	6.0	3.0	400
0.70	138	9.0	4.0	550	6.0	3.0	400
2.07	45	11.0	4.8	397	6.9	3.1	192
1.88	49	20.0	3[illegible]	333	16.0	1[illegible]	542

COMPARISON OF SIRUPS MADE FROM INOCULATED SAP AND FROM NATURAL SOUR SAP

The reader with a practical turn of mind may well inquire how the quality of the sirups made from the inoculated saps compares with that of the sirups made from saps which have become sour from natural causes. It might seem that the artificial introduction of large numbers of bacteria into sap would result in the production of an abnormal sirup, far inferior to that secured from saps souring under natural, and perhaps unavoidable, conditions; but such is not the fact. Much sirup and sugar are annually made from sap more seriously injured by natural souring than was most of that discussed in the foregoing pages. The organisms at work are the same in either case, but the conditions under which souring occurs in nature are as a rule more favorable to bacterial growth than were those maintained in the incubator. In nature, however, there is almost always a mixed infection, in which the predominant forms are those most seriously injurying the color. In the incubator it was possible so to control conditions as to give the advantage to particular groups at will, so that in some cases the flavor of the sirup was more seriously injured than is likely to occur in nature unless the conditions are extremely bad. The writer, while unwilling to acknowledge himself a great offender, has personally made sirups for commercial sale from saps injured more seriously by natural souring than were most of those employed in these experiments. In fact only the most painstaking care can prevent the occurrence of such injury towards the close of the season.

The facts are brought out clearly by a study of the data already presented. Twelve of the sirups discussed were made from sap which had soured naturally. The average color was 8.3, flavor 4.3, and score 381; average depreciation from first run control, color 9.2, flavor 2.6, and score 504. Three of these samples, however, (Nos. 84, 85 and 86) were made from sour sap which had been allowed to stand two or three days in the buckets so that it was very poor indeed; and six (Nos. 88, 121, 123, 125,

127 and 130) were made from sour sap drawn very late in the season, so that it was not only sour but buddy. The three remaining samples (Nos. 22, 23 and 25), however, were made from sour sap free from buddy flavor. Samples 22 and 25 were concentrated to sirup the same day they flowed, and at the time of evaporation the first drops to run had not been out of the vascular tissue more than twelve hours. Sample 23 was evaporated thirty-six hours after it began to run. These sirups might be expected to be at least as good as the ordinary material made from sour sap and probably superior to it. They are therefore used in the following comparison of depreciation values, which values in these cases are calculated from the average of all controls of the three seasons, instead of from the average of first run controls. The average color was 12, flavor 3, and score 600; average depreciation from all controls, color 8.2, flavor 1.6, and score 311.

DISCUSSION OF DEPRECIATION VALUES

The specific influence of the various groups of organisms and of natural souring upon sirup is seen most clearly from a study of the depreciation values. If the groups are arranged according to the average amounts of depreciation in color they fall into the following order:

TABLE 12. ORDER OF AVERAGE DEPRECIATION IN COLOR

	Group	Depreciation Color	Depreciation Flavor	Depreciation Score
1	Non-fluorescent bacteria,	0.5	1.5	100
2	Incubator control,	1.8	0.7	87
3	*B. aceris*,	2.7	2.0	242
4	Pink coccus,	2.8	1.6	171
5	Composites,	3.3	1.5	213
6	Failures,	3.4	0.3	103
7	Gray yeast,	3.8	1.7	234
8	Red yeast,	4.1	1.7	215
9	Green mold,	4.3	2.0	281
10	Fluorescent bacteria plus spore-bearers,	4.4	2.6	307
11	Fluorescent bacteria,	4.8	1.6	242
12	*Natural sour sap*,	*8.2*	*1.6*	*311*

A glance at the foot of the table shows that the sirups most seriously injured in color were made from sap souring naturally.

The sirups showing the next most serious depreciation in color were those inoculated with green fluorescent bacteria. Then comes a group in which fluorescent organisms were employed in the inoculation and developed in association with such spore-bearers as were present. It has already been shown that the fluorescent bacteria exercised some influence on the sirups of the next five groups also, while in the remaining groups they were either absent or present in smaller numbers for a shorter time.

If the depreciation figures are arranged according to flavor values a different order is secured, as follows:

TABLE 13. ORDER OF AVERAGE DEPRECIATION IN FLAVOR

	Group	Depreciation Color	Depreciation Flavor	Score
1	Failures,	3.4	**0.3**	103
2	Incubator control,	1.8	**0.7**	87
3	Non-fluorescent bacteria,	0.5	**1.5**	100
4	Composites,	3.3	**1.5**	213
5	Pink coccus,	2.8	**1.6**	171
6	Fluorescent bacteria,	4.8	**1.6**	242
7	*Natural sour sap,*	8.2	*1.6*	*311*
8	Gray yeast,	3.8	**1.7**	234
9	Red yeast,	4.1	**1.7**	215
10	*B. aceris,*	2.7	**2.0**	242
11	Green molds,	4.3	**2.0**	281
12	Fluorescent bacteria plus spore-bearers,	4.4	**2.6**	307

The spore-bearing bacteria, green molds, *B. aceris,* both groups of yeasts and the natural sour material, occupy positions in this table below the fluorescent group. The sirups of five of these six groups were influenced somewhat in quality by the fluorescent bacteria, and it is probable that the contrasts in quality are not so great as they would have been had the fluorescent bacteria been entirely eliminated; yet it still appears perfectly clear that the organisms which most seriously injure the flavor of maple sirup are not those producing the darker grades of color.

If the groups are re-arranged in the order of depreciation in score, an idea may be gained of their relative commercial values.

TABLE 14. ORDER OF AVERAGE DEPRECIATION IN SCORE

	Group	Depreciation Color	Depreciation Flavor	Score
1	Incubator control,	1.8	0.7	87
2	Non-fluorescent bacteria,	0.5	1.5	100
3	Failures,	3.4	0.3	103
4	Pink coccus,	2.8	1.6	171
5	Composites,	3.3	1.5	213
6	Red yeast,	4.1	1.7	215
7	Gray yeast,	3.8	1.7	234
8	Fluorescent bacteria,	4.8	1.6	242
9	*B. aceris*,	2.7	2.0	242
10	Green molds,	4.3	2.0	281
11	Fluorescent bacteria plus spore-bearers,	4.4	2.6	307
12	*Natural sour sap*,	5.2	1.6	311

DISCUSSION OF LAST RUN SIRUPS

Examination of the statistical tables shows that the dark color of late run sirup is to be attributed to the action of micro-organisms, since the sirups made from last run material gathered without infection were always of a light hue. The average depreciation in color as calculated upon the first run controls was 0.8 point. At first sight it would appear that the results as applied to flavors lack uniformity. The sirups of the first season suffered no depreciation in flavor while those of the last two seasons uniformly fell off three points; but, as has been already suggested, this result is accounted for by the meteorological divergencies of the several seasons (pages 328 and 380).

INFLUENCE OF INERT EXTRANEOUS MATERIAL

Before formulating answers to the questions propounded at the outset, attention is called to the fact that in these studies no attention has been paid to the influence upon the color and flavor of sirup of inert extraneous matter, such as colored rain water, bark, insects, caramel and the like. Everyone recognizes the importance of eliminating such material, but the following paragraph showing the influence of caramel may be worthy of record.

The commercial supply of sap from the sugar place where the inoculation experiments were carried out was concentrated

in a multiple pan evaporator in which the sirup was always drawn off from the rear pan. Of course the usual accumulation of the so-called niter (mainly malate of lime) occurred in the sirup pan. During the height of the season the capacity of the equipment was hardly sufficient for the number of buckets hung, so that in order to save time on one occasion the daily cleansing of the pan was omitted. A decided darkening was soon noted in the grade of sirup obtained and suspicion was directed towards the sirup pan. The fire was banked and the niter removed. An improvement of several points in color of the sirup immediately resulted, showing that the sugar mechanically included in the niter was being caramelized and was exercising a detrimental influence. After this the color of the sirup was carefully watched, and whenever a depreciation in color began to appear the niter was removed from the sirup pan and the former light light color of the sirup was restored.

CONCLUSIONS

Returning now to the questions these studies were inaugurated to answer, the work has shown that the depreciation in color and flavor of maple sirup which ordinarily occurs in the commercial sugar place as the season progresses is to be attributed to the action of micro-organisms. Certain groups exercise a more detrimental influence upon color than upon flavor, while with other groups the reverse is true. The influence of each group appears more or less specific and characteristic. The most common form of organisms present in maple sap, the fluorescent bacteria, injures the flavor much less than it does the color. Those organisms which most seriously affect the flavor of sirup, the non-fluorescent, spore-bearing bacteria, molds, and stringy sap organisms, do not seriously darken the color. They do, however, frequently render the sirup cloudy and so viscid that it does not clear perfectly, even if left undisturbed for months.

In addition to the depreciation due to bacteria there may occur deterioration due to physiological changes in the tree itself.

PLATE V.—A jar of normal sap compared with two jars containing an infected sap of the so called milky type. (See pages 334-335).

PLATE VI. Colonies developing on nutrient agar and synthetic agar, respectively, from one-twentieth of a cubic centimeter of maple sap No. 66 received in tin buckets. (See pages 343, 373.)

In certain seasons these changes which produce the "buddy" flavor may not occur in Vermont until after the sap flow ceases. In ordinary years, however, the season is interrupted by periods of growing weather, so that the vegetative activity of the tree is resumed some time before the final discontinuance of night freezes, and the influence of these physiological changes becomes manifest in the true "buddy" flavor which appears in the sirup. Formerly the opinion was commonly held that this depreciation was due to the presence of a relatively large proportion of invert sugar. An examination of the analytical data clearly demonstrates that this view is erroneous. There is a decline rather than an increase in the content of invert sugar as the season advances. The exact nature of the cause of the true buddy flavor is unknown. In this connection, attention is again directed to the statement on page 346, to the effect that frequently the so-called buddy material is not buddy in the sense in which the term is employed in this bulletin. Much sirup popularly but erroneously termed buddy possesses objectionable foreign flavors due solely to the action of micro-organisms.

REMEDIAL MEASURES

Practical remedial measures must be based upon efforts to minimize the contamination with micro-organisms and to restrict the period of their action to the shortest possible time. The lower their content and the shorter their period of growth, the better the product. As in dairying, cleanliness must be the watchword of the producer of superior goods. Clean spouts, clean and covered buckets, and clean holders are necessities. The use of metal utensils is to be preferred to the employment of wooden ones, because the latter material affords organic matter upon which organisms may develop. Moreover wooden utensils are less readily cleaned. Covered buckets are preferable to open ones, not only because they keep out rain and snow, but because they prevent the entrance of bits of falling bark, decayed wood, and other inert matter. Such material is always heavily charged

with bacteria and other organisms, so that in addition to the coloring matter carried in the refuse itself, agents are introduced which further discolor the product through their vital activities.

The practice of storing sap is one to be avoided whenever possible. Modern evaporators not only make long periods of boiling unnecessary, but they make it possible to concentrate the runs day by day as they occur. They are doubtless important factors contributing to the improved quality of evaporator sirup as compared with that produced by older methods. When storage is resorted to, the temperature of the tank should be kept as low as possible, because the lower the temperature the slower the micro-organic development. Holders should be located without rather than within the boiling house, where the heat of the pans will not influence their temperature.

The evidence obtained indicates that the sugar-maker cannot expect to produce a high quality of sirup at the close of the season in average years, because there is no known means by which the physiologically induced "buddy" flavor may be avoided. So long as the depreciation is caused solely by bacteria, cleanly methods will enable the producer to maintain a high standard of excellency in his product; but if the physiological activity of the tree begins to be manifest, the producer will find himself unable to manufacture an article of high excellency as regards flavor. The light color can be maintained indefinitely, but the "buddy" flavor is so objectionable that the market value of the sirup is insufficient to render its production profitable.

PART II

DISCUSSION OF PHYSICAL AND CHEMICAL DATA SECURED ON MAPLE SIRUPS OBTAINED FROM SAPS INOCULATED WITH MICRO-ORGANISMS.

BY C. H. JONES

The analyses of maple sirup displayed in tables 15-37 (pages 420 to 457) were made on samples secured in the prosecution of the work discussed in the preceding pages. They were obtained during three successive sugar seasons and include not only pure sirups made under the most favorable conditions possible, but, also, those representing the extremes of both natural and artificial inoculation with bacteria, yeasts and molds.

The samples were sterilized at the time of manufacture and stored in the dark in sealed half-pint "lightning" jars. All analyses were made immediately after opening the jars. Particular attention was paid to the sucrose and invert sugar contents, but, in addition, the moisture, ash, and malic acid value were secured in order to determine the effect of the treatment on the data usually employed in judging the purity of maple products. The analytical methods employed were those in ordinary use, as outlined in bulletin 134 of the Bureau of Chemistry of the United States Department of Agriculture.

It should be remembered that these samples were all pure maple sirups so far as admixture with cane or other sugars is considered. They were, however, either naturally or artificially inoculated with the types of micro-organisms normally present in maple sap. This inoculation, as will be shown, has a greater influence on color and flavor (physical characteristics) than on the chemical composition.

A total of 128 samples were examined. The analytical results are reported in groups, based mainly on the nature of the inoculating organisms employed. The sample numbers and groupings correspond to those outlined on pages 402 to 409.

Before beginning a detailed discussion, attention should be called to a few general conditions obtaining in the manufacture and analysis of the sirups, which doubtless to some extent affected the chemical data.

1. All samples were secured from the same sugar orchard.

2. They were made during three successive sugar seasons.

3. The size of the sample and the manner of its evaporation made it difficult closely to control the end boiling point and, consequently, large individual and yearly variations were observed in the concentration of the sirups, as is shown below.

TABLE 15. MOISTURE CONTENTS OF SIRUP SAMPLES

Number of samples secured	Year	Average	Maximum	Minimum	Extreme yearly variation
		%	%	%	%
26	1909	33.67	38.88	29.44	9.44
60	1910	38.01	47.60	32.46	15.14
42	1911	30.39	38.97	26.76	12.21

The average obtained in 1909 more nearly approaches the eleven-pound per gallon standard,[1] which calls for a moisture content of from 34 to 35%, than do those secured in the two following years. The 1910 samples were nearly all light weight goods, with an extreme variation of 15.14% in moisture. The average for 1911 indicates that most of the samples were considerably more concentrated than the eleven-pound standard[1] requires. A thermometer was used to determine the density during manufacture, but, unfortunately, due allowance was not made for the boiling points of different grades of sirup. These figures will be referred to later during the discussion of the ash data (pages 464 and 465).

No particular care was taken to remove the "niter" in the laboratory previous to the analysis of the 1909 samples, Nos. 1-26. It was thoroughly removed from the 102 samples of the two succeeding seasons by sedimentation during a period of seven months.

[1] Vt. Sta. Bul. 26 (1891); Rpt. 18, p. 331 (1905); U. S. Dept. Agr., Bu. Chem., Bul. 134, pp. 74-75 (1910).

EXPLANATION OF PHYSICAL AND CHEMICAL DATA SHOWN IN TABLES 16 TO 34

The chemical analysis of each sample was preceded by a careful physical examination, for the details of which see pages 345 to 349. The color was determined after the standard of Bryan[1] which comprises twenty grades. (See page 349). The first grade is never obtained in practical manufacture. As commercially rated for average crops, any grade below 8 may be considered No. 1, from 8 to 11 inclusive No. 2, and from 12 to 15 inclusive No. 3.

The sirups were grouped in 6 classes as regards flavor. No. 1 corresponding to prime, 2 good, 3 medium, 4 poor, 5 buddy, and 6 buddy and rank. A certain few sirups grading good and medium, were designated 2^1 and 2^2, and similarly a few, falling between medium and poor, were rated 3^1. A flavor of 5 or 6 would immediately condemn the sample for commercial purposes.

The scoring system adopted for these sirups is based on the flavor and color as explained on pages 346 to 349.

The column headed "undetermined" is obtained by subtracting the sum of the moisture, sucrose, invert sugar, ash and malic acid value from 100. It is, of course, affected by all the errors in the five determinations mentioned, and includes proteids, tannin, organic acids and other non-sugars.

GROUP 1. *Controls.* As the word "control" indicates, these samples were prepared from sap selected to serve as checks on experimental procedure. The first two lots, Nos. 6 and 20, were not boiled fresh, but were held in the cold for about 3 days during the incubation period. All the other saps in this group were strictly fresh when boiled.

The resulting sirups were of fine flavor and light in color. No. 49 is credited with the highest score possible, 975, while 5 more of the 11 lots are hardly inferior to No. 49. They would all be classed as of the highest grade.

[1] U. S. Dept. Agr., Bu. Chem., Bul. 134, p. 15 (1910).

TABLE 16. MAPLE SIRUP. CONTROLS

Sample number	Color	Flavor	Score	Moisture	Sucrose	Invert sugar	Total ash	Soluble ash	Insoluble ash	Malic acid value	Undetermined
				%	%	%	%	%	%	%	%
6	6	1	875	33.99	62.98	0.64	0.62	0.27	0.35	0.61	1.16
20	6	1	875	33.54	63.38	1.05	0.83	0.25	0.58	1.04	0.16
27	4	2	875	34.46	63.04	0.20	0.49	0.34	0.15	0.48	1.33
41	4	1	925	39.78	58.46	0.62	0.46	0.22	0.24	0.53	0.15
49	2	1	975	38.39	59.46	0.26	0.50	0.24	0.26	0.51	0.88
58	3	1	950	36.30	61.80	0.28	0.47	0.28	0.19	0.53	0.62
68	4	1	925	37.33	60.73	0.32	0.42	0.28	0.14	0.51	0.69
89	3	2	900	29.96	67.11	0.24	0.43	0.22	0.21	0.73	1.53
97	3	1	950	30.36	66.88	0.13	0.47	0.30	0.17	0.51	1.65
105	3	2	900	30.53	66.85	0.17	0.55	0.38	0.17	0.42	1.48
113	4	2	875	29.99	67.89	0.43	0.53	0.35	0.18	0.51	0.65
Average,	3.8	1.4	911	34.06	63.51	0.39	0.52	0.28	0.24	0.57	0.95
Max.,	6	2	975	39.78	67.89	1.05	0.83	0.38	0.58	1.04	1.65
Min.,	2	1	875	29.96	58.46	0.13	0.42	0.22	0.14	0.42	0.15

MAPLE SIRUP. CONTROLS

Calculated to a moisture-free basis

Sample number	Sucrose	Invert sugar	Total ash	Soluble ash	Insoluble ash	ALKALINITY: Soluble ash	ALKALINITY: Insoluble ash	Malic acid value	Undetermined	Date
	%	%	%	%	%			%	%	
6	95.41	0.97	0.94	0.41	0.53	36	94	0.92	1.76	3-24-09
20	95.37	1.58	1.25	0.38	0.87	45	129	1.56	0.24	4- 1-09
27	96.19	0.30	0.75	0.53	0.22	58	71	0.73	2.03	3- 7-10
41	97.08	1.03	0.77	0.37	0.40	59	85	0.88	0.24	3-14-10
49	96.51	0.42	0.81	0.39	0.42	52	85	0.83	1.43	3-20-10
58	97.01	0.43	0.73	0.43	0.30	58	113	0.83	1.00	3-21-10
68	96.90	0.51	0.68	0.45	0.23	54	56	0.81	1.10	3-22-10
89	95.81	0.34	0.61	0.32	0.29	44	53	1.01	2.23	3-22-11
97	96.04	0.19	0.68	0.43	0.25	56	49	0.73	2.36	3-26-11
105	96.23	0.24	0.79	0.54	0.25	58	48	0.61	2.13	3-27-11
113	96.97	0.61	0.75	0.50	0.25	62	58	0.73	0.94	3-30-11
Av'ge,	96.32	0.60	0.77	0.41	0.36	51	77	0.86	1.45	
Max.,	97.08	1.58	1.25	0.54	0.87	62	129	1.56	2.36	
Min.,	95.37	0.19	0.61	0.32	0.22	36	48	0.61	0.24	

The average water content for the group is 34.06%. This figure is about normal, yet individual sirups vary from 30.78 to 29.96%. The sucrose shows the usual variations depending on the moisture content, ranging from 58.46 to 67.89, and averaging 63.51%. When calculated to a dry matter basis these large variations are eliminated and the average sucrose content becomes 96.32%. The invert sugar varies from 0.13 to 1.05, averaging 0.39%. Investigation has shown that there are but very small amounts of invert sugar in the sap of the maple as it comes from the tree, and, consequently, if quickly and properly concentrated, the resulting sirup should carry only a small amount. When the invert sugar in maple sirup calculated to a moisture free basis is less than 0.60%, it should not be attributed to excessive micro-organic infection. No. 20 was obtained about the middle of the season of 1909 from sap held 3 days in the cold; and its invert sugar percentage, 1.05, would indicate that the sucrose had suffered a slight decomposition.

The minimum ash and malic acid value figures established by the writer for pure Vermont maple sirup of standard weight and density, together with similar data obtained by Bryan for Vermont as well as for the United States[2], are shown in the following table:

TABLE 17. MINIMUM ASH AND MALIC ACID VALUES FOR PURE MAPLE SIRUP
Calculated to a moisture-free basis

	Jones [1] Vermont samples	Bryan [2] Vermont samples	Bryan [2] United States samples
Total ash,	0.77%	0.77%	0.68%
Insoluble ash,	0.23%	0.23%	0.23%
Malic acid value,	0.61%	0.58%	0.31%

The agreement on the Vermont samples is very close. The Jones standard was secured on samples made in 1904 and the Bryan results on those obtained in 1909. In both cases they are based on about 50 samples representing all grades of pure

[1] Vt. Sta. Rpt. 18, p. 334 (1905).
[2] U. S. Dept. Agr., Bu. Chem., Bul. 134, pp. 74-75 (1910).

sirup. The United States minimum is based on 395 samples, representing all the important maple sugar producing sections. Attention is called to the fact that the minimum for insoluble ash for the entire United States is identical with the figure twice obtained by different investigators on Vermont samples.

The ash and malic acid value data of the sirups in this and subsequent groups will be discussed primarily with a view of comparing the data with the standards just enumerated. It should be remembered that many of the samples discussed in the following groups are exceptional as to inoculation and manufacture. Such divergencies from the normal as were found will be noted and reasons assigned for the deficiencies.

The average total and insoluble ash contents of the group on a moisture free basis are 0.77 and 0.36% respectively, with variations for total ash from 0.61 to 1.25 and for insoluble ash from 0.22 to 0.87%. While the average of these 11 sirups is normal as regards standards, yet in six cases the total ash is below 0.77% (the Vermont standard) and in one case below the United States minimum. The insoluble ash runs 0.01% low in one instance, but in all others equals or exceeds the 0.23% figure.

The malic acid value meets the standard in every instance, the average figure obtained being 0.86%, with extremes of 1.56 and 0.61%.

Attention is here called to the fact that, excepting Nos. 27, 58 and 68, the samples showing a low total ash had a relatively small water content, and that in all cases the "niter" had been thoroughly removed by a full seven months' sedimentation. The deficiencies occurring during 1910 and 1911, if considered in connection with color, flavor, and the remaining ash and malic acid data, would not embarrass the analyst familiar with maple products.

The undetermined column shows variations from 0.24 to 2.36, averaging 1.45%, which is about the usual amount found in the ordinary run of good grade maple sirups.

Group 2. *Incubator controls.* The saps from which these sirups were made were kept during the incubation period, generally not over 3 days, before being concentrated. They were in many cases probably slightly contaminated from natural sources and should, therefore, be considered only as of ordinary quality. Sample 43 remained clear for 2 days, but showed a milky bacterial growth the third day before boiling. No. 65 likewise soured early and made a sirup of poor quality. All the samples graded well as to color and in only two cases was the flavor considered medium or poor.

TABLE 18. MAPLE SIRUP. INCUBATOR CONTROLS

Sample number	Color	Flavor	Score	Moisture	Sucrose	Invert sugar	Total ash	Soluble ash	Insoluble ash	Malic acid value	Undetermined
				%	%	%	%	%	%	%	%
1	7	1	850	38.52	59.54	0.66	0.61	0.24	0.37	0.65	0.02
34	6	2[1]	800	36.06	60.97	0.27	0.90	0.38	0.52	0.91	0.89
38	5	2[1]	825	38.37	58.45	0.30	0.71	0.34	0.37	0.62	1.55
40	9	2	750	34.89	61.89	0.42	0.63	0.25	0.38	0.59	1.58
43	7	2	800	40.03	56.39	1.58	0.53	0.25	0.28	0.53	0.94
45	4	1	925	42.19	55.66	0.30	0.52	0.24	0.28	0.60	0.73
56	5	2	850	37.92	59.61	0.84	0.56	0.34	0.22	0.46	0.61
57	2	1	975	40.65	57.26	0.36	0.48	0.25	0.23	0.53	0.72
65	6	4	625	42.03	53.27	2.04	0.60	0.25	0.35	0.63	1.43
96	5	2	850	27.81	68.82	0.32	0.51	0.33	0.18	0.42	2.12
104	4	2	875	29.46	68.42	0.29	0.49	0.33	0.16	0.41	0.93
112	5	2	850	31.34	67.01	0.76	0.49	0.35	0.14	0.31	0.09
120	5	3	775	38.97	58.06	0.35	0.44	0.28	0.16	0.44	1.74
Average,	5.4	2.0	827	36.78	60.41	0.65	0.57	0.29	0.28	0.55	1.04
Max.,	9	1	975	42.19	68.82	2.04	0.90	0.38	0.52	0.91	2.12
Min.,	2	3	625	27.81	53.27	0.27	0.44	0.24	0.14	0.31	0.02

MAPLE SIRUP. INCUBATOR CONTROLS

Calculated to a moisture-free basis

Sample number	Sucrose	Invert sugar	Total ash	Soluble ash	Insoluble ash	ALKALINITY		Malic acid value	Undetermined	Date
						Soluble ash	Insoluble ash			
	%	%	%	%	%			%	%	
1	96.84	1.07	0.99	0.39	0.60	55	120	1.06	0.04	3-24-09
34	95.36	0.42	1.41	0.59	0.82	69	153	1.42	1.39	3- 7-10
38	94.80	0.48	1.15	0.55	0.60	74	119	1.00	2.57	"
40	95.05	0.64	0.96	0.38	0.58	62	107	0.91	2.44	"
43	94.19	2.64	0.88	0.41	0.47	58	88	0.88	1.41	3-14-10
45	96.28	0.51	0.89	0.41	0.48	60	96	1.04	1.28	"
56	96.02	1.35	0.90	0.54	0.36	58	95	0.73	1.00	3-20-10
57	96.47	0.60	0.81	0.52	0.39	61	81	0.90	1.22	"
65	91.89	3.52	1.03	0.43	0.60	60	122	1.09	2.47	3-21-10
96	95.33	0.44	0.71	0.46	0.25	55	49	0.65	2.87	3-22-11
104	97.00	0.41	0.69	0.47	0.22	62	42	0.60	1.30	3-26-11
112	97.60	1.11	0.71	0.50	0.21	59	47	0.44	0.14	3-27-11
120	95.12	0.57	0.71	0.45	0.26	66	75	0.72	2.88	3-30-11
Average,	95.55	1.04	0.90	0.46	0.44	61	92	0.87	1.64	
Max.,	97.60	3.52	1.41	0.59	0.82	74	153	1.42	2.87	
Min.,	91.89	0.41	0.69	0.38	0.21	55	42	0.44	0.04	

The moisture content in these thirteen samples varies from 27.81 to 42.19 with an average of 36.78%.

The sucrose ranges from 53.27 to 68.82 and averages 60.41%. No. 65, which contains the lowest sucrose figure, is in consequence high in both water and invert sugar.

The invert sugar averages 0.65% with a mixinum of 2.04 and a minimum of 0.27%. None of the samples, save Nos. 43 and 65, show marked evidences of souring previous to boiling. No. 1 was purposely evaporated at a slow rate, about 6 hours, or four times the usual interval, being taken. Its low invert sugar content, 0.66%, together with its satisfactory flavor and color, indicate that the time taken in boiling this fairly pure sap produced no marked inversion effect on the sucrose.

Four of these samples, Nos. 96, 104, 112 and 120, when calculated to a moisture free basis, show a deficiency of total ash, two of which, Nos. 104 and 112, are just under the limit for insoluble ash, while one, No. 112, is low in malic acid value. In three of these four cases the concentration of the sirup was carried too far, the moisture content ranging from 27.81 to 31.34%. The malic acid value figure, 0.44%, is the lowest ever observed by the writer on pure Vermont goods.

GROUP 3. *Inoculated with non-fluorescent bacteria.* These organisms are common in nature. When they occur in the sap and are allowed to develop, they injure the flavor more than they do the color of the resulting sirup. Two of the four samples were rated medium as to flavor, and two, good. The color of all four was exceptionally light, easily being classed No. 1 as commercially graded.

TABLE 19. MAPLE SIRUP. INOCULATED WITH NON-FLUORESCENT BACTERIA

Sample number	Color	Flavor	Score	Moisture	Sucrose	Invert sugar	Total ash	Soluble ash	Insoluble ash	Malic acid value	Undetermined
				%	%	%	%	%	%	%	%
9	5	2	850	33.26	63.81	0.52	0.57	0.26	0.31	0.47	1.37
10	6	3	750	32.21	64.98	0.52	0.78	0.27	0.51	0.60	0.91
12	6	3	750	36.35	60.92	1.42	0.76	0.27	0.49	0.73	—.18
55	5	2	850	36.97	59.73	1.50	0.63	0.26	0.37	0.48	0.69
Average,	5.5	2.5	800	34.70	62.36	0.99	0.69	0.27	0.42	0.57	0.69
Max.,	6	3	850	36.97	64.98	1.50	0.78	0.27	0.51	0.73	1.37
Min.,	5	2	750	32.21	59.73	0.52	0.57	0.26	0.31	0.47	—.18

MAPLE SIRUP. INOCULATED WITH NON-FLUORESCENT BACTERIA

Calculated to a moisture-free basis

Sample number	Sucrose	Invert sugar	Total ash	Soluble ash	Insoluble ash	ALKALINITY		Malic acid value	Undetermined	Date
						Soluble ash	Insoluble ash			
	%	%	%	%	%			%	%	
9	95.61	0.78	0.85	0.39	0.46	51	117	0.70	2.06	3-27-09
10	95.86	0.77	1.15	0.40	0.75	35	145	0 88	1.34	"
12	95.71	2.23	1.19	0.42	0.77	41	145	1.14	—.27	"
55	94.76	2.38	1.00	0.40	0.60	57	123	0.75	1.11	3-20-10
Average,	95.50	1.52	1.06	0.42	0.64	46	132	0.87	1.05	
Max.,	95.86	2.38	1.19	0.42	0.77	57	145	1.14	2.06	
Min.,	94.76	0.77	0.85	0.39	0.46	35	117	0.70	—.27	

The moisture content of these samples was quite uniform, a variation of less than 5% being found. Its average was 34.70%.

The sucrose varied from 59.73 to 64.98, averaging 62.36%.

The invert sugar, while averaging higher than in the previously mentioned controls, did not show such extremes. The average of 0.99% as analyzed, equivalent to 1.52% on a dry basis, is clearly indicative of the fact that the organisms used were not active inverters of sucrose. The records show that the sap from which sirup 55 was made had a bacterial count of nearly 3,000,000. While apparently inactive as regards inversion they did, however, affect the flavor of the product, causing it to fall off 1.5 points from the control.

The ash and malic acid value data in all cases exceed the usual standard. It should be noted, however, that the first three samples, Nos. 9, 10 and 12, were from the 1909 crop, and, as has been mentioned, no particular care was taken to remove all niter as was the case with No. 55.

Group 4. *Inoculated with pink cocci.* The presence of these organisms in maple sap is decidedly detrimental to the production of a first class sirup. They seriously affect the flavor but have no marked influence on the color.

TABLE 20. MAPLE SIRUP. INOCULATED WITH PINK COCCI

Sample number	Color	Flavor	Score	Moisture	Sucrose	Invert sugar	Total ash	Soluble ash	Insoluble ash	Malic acid value	Undetermined
				%	%	%	%	%	%	%	%
46	6	2	825	38.42	58.55	0.65	0.63	0.27	0.36	0.60	1.15
47	6	3	750	40.64	52.72	4.69	0.60	0.27	0.33	0.52	0.83
50	6	2[1]	800	40.03	56.17	1.32	0.50	0.23	0.27	0.50	1.48
51	4	2	875	37.65	60.67	0.41	0.51	0.31	0.20	0.49	0.27
107	5	4	600	27.90	68.15	0.50	0.51	0.36	0.15	0.42	2.52
54	7	2[2]	750	38.30	58.07	1.67	0.51	0.27	0.24	0.44	1.01
Average,	5.7	2.6	775	37.15	59.05	1.54	0.54	0.28	0.26	0.50	1.22
Max.,	7	4	875	40.64	68.15	4.69	0.63	0.36	0.36	0.60	2.52
Min.,	4	2	600	27.90	52.72	0.41	0.50	0.23	0.15	0.42	0.27

MAPLE SIRUP. INOCULATED WITH PINK COCCI

Calculated to a moisture-free basis

Sample number	Sucrose	Invert sugar	Total ash	Soluble ash	Insoluble ash	ALKALINITY		Malic acid value	Undetermined	Date
						Soluble ash	Insoluble ash			
	%	%	%	%	%			%	%	
46	95.07	1.05	1.02	0.44	0.58	162	113	0.97	1.89	3-14-10
47	88.81	7.90	1.01	0.46	0.55	64	107	0.88	1.40	"
50	93.83	2.20	0.83	0.38	0.45	62	95	0.83	2.31	3-20-10
51	97.30	0.65	0.82	0.50	0.32	63	73	0.79	0.44	"
107	94.52	0.69	0.71	0.50	0.21	57	34	0.58	3.50	3-27-11
54	94.11	2.71	0.82	0.43	0.39	56	75	0.71	1.65	3-20-10
Average,	93.94	2.45	0.86	0.45	0.41	61	83	0.79	1.96	
Max.,	97.30	7.90	1.02	0.50	0.58	64	113	0.97	3.50	
Min.,	88.81	0.65	0.71	0.38	0.21	56	34	0.58	0.44	

The moisture content of the six sirups averaged 37.15 with extremes of 40.64 to 27.99%. The minimum amount is found in No. 107, the only 1911 sample in this series.

The sucrose averages 59.05 and the extremes are 52.72 and 68.15%. The sample, No. 47, with the highest moisture content carries the lowest sucrose percentage. The relatively large amount of invert sugar present is also a contributing factor.

The invert sugar shows a surprising variation of from 0.41 to 4.69 with an average of 1.54%. This maximum figure, in No. 47, when calculated to the moisture free basis is equivalent to 7.99%. This result may be attributed to the action on the sucrose of a certain strain of pink coccus. Other strains did not give as positive evidences of sucrose inversion.

No. 107, as compared with standard, is slightly deficient in total and insoluble ash and in malic acid value.

Group 5. *Failures.* The saps from which the sirups thus characterized were made, were variously inoculated, but the introduced organisms could not be recovered at the close of the incubation period. The natural infection may have suppressed their development or the inoculating organisms may have been killed by those normally present. Whatever the infection, it had no marked influence upon the results in the invert sugar column.

The color averaged a little poorer in these samples than in those previously mentioned under the head "incubator control," while flavor showed a slight gain.

TABLE 21. MAPLE SIRUP. FAILURES

Sample number	Color	Flavor	Score	Moisture	Sucrose	Invert sugar	Total ash	Soluble ash	Insoluble ash	Malic acid value	Undetermined
				%	%	%	%	%	%	%	%
31	6	2	825	38.21	59.70	0.19	0.70	0.34	0.36	0.34	0.86
32	6	2	825	37.37	60.07	0.29	0.71	0.25	0.46	0.65	0.91
33	7	2	800	35.96	61.23	0.15	0.76	0.34	0.42	0.64	1.26
36	9	2[1]	725	37.52	60.08	0.38	0.65	0.27	0.38	0.45	0.92
37	11	2[1]	675	34.43	62.87	0.41	0.73	0.24	0.49	0.65	0.91
39	7	2	800	36.34	61.23	0.17	0.71	0.22	0.49	0.62	0.93
106	7	3	725	30.48	67.64	0.46	0.48	0.33	0.15	0.41	0.53
108	4	3	800	29.64	67.10	0.63	0.46	0.32	0.14	0.35	1.82
Average,	7.0	2.3	772	34.99	62.48	0.33	0.65	0.29	0.36	0.52	1.03
Max.,	11	3	825	38.21	67.64	0.63	0.76	0.34	0.49	0.65	1.82
Min.,	6	2	675	29.64	59.70	0.15	0.46	0.22	0.14	0.34	0.53

MAPLE SIRUP. FAILURES

Calculated to a moisture-free basis

Sample number	Sucrose	Invert sugar	Total ash	Soluble ash	Insoluble ash	ALKALINITY: Soluble ash	ALKALINITY: Insoluble ash	Malic acid value	Undetermined	Date
	%	%	%	%	%			%	%	
31	96.62	0.30	1.14	0.55	0.59	62	133	0.55	1.39	3- 7-10
32	95.91	0.46	1.13	0.39	0.74	54	102	1.03	1.47	"
33	95.61	0.23	1.18	0.52	0.66	62	151	0.99	1.99	"
36	96.16	0.61	1.06	0.44	0.62	57	105	0.73	1.44	"
37	95.88	0.64	1.11	0.36	0.75	51	79	0.99	1.38	"
39	96.18	0.26	1.12	0.35	0.77	67	120	0.97	1.47	"
106	97.30	0.66	0.69	0.47	0.22	58	56	0.59	0.76	3-27-11
108	95.37	0.90	0.65	0.45	0.20	58	37	0.49	2.59	"
Average,	96.13	0.51	1.00	0.45	0.55	59	98	0.80	1.56	
Max.,	97.30	0.90	1.18	0.55	0.77	67	151	1.03	2.59	
Min.,	95.37	0.23	0.65	0.35	0.20	51	37	0.49	0.76	

The moisture in the sirups of this group varies from 29.64 to 38.21, and averages 34.99%.

The sucrose averages 62.48%, equivalent, on a moisture free basis, to 96.13%.

The invert sugar percentages were low, the average on a moisture free basis being 0.51%, the highest, 0.90, the lowest 0.23%. This checks closely with the results shown in tables 16 and 18, with which the data secured with the samples of this group are comparable.

The total and insoluble ash contents are low of standard in two of the eight samples and the malic acid value is deficient in three samples.

Group 6. *Inoculated with red yeasts.* These organisms are quite common, particularly toward the close of the season. The evaporating sap and sirup containing them give off an offensive yeasty odor. They seriously impair the flavor and in many instances cause the color to grade several shades darker than it otherwise would; and they also materially increase the invert sugar content because of their action on the sucrose.

TABLE 22. MAPLE SIRUP. INOCULATED WITH RED YEASTS

Sample number	Color	Flavor	Score	Moisture	Sucrose	Invert sugar	Total ash	Soluble ash	Insoluble ash	Malic acid value	Undetermined
				%	%	%	%	%	%	%	%
14	10	2	725	33.17	63.69	2.01	0.87	0.31	0.56	0.59	−0.33
62	9	4	550	40.18	53.40	4.37	0.51	0.22	0.29	0.49	1.05
15	8	2	750	38.88	57.00	1.82	0.77	0.26	0.51	0.92	0.61
114	5	3	775	30.69	66.75	0.70	0.49	0.34	0.15	0.39	0.98
18	10	2	725	30.17	64.00	2.18	0.72	0.27	0.45	0.72	2.21
35	12	2	650	32.66	64.06	0.77	0.64	0.25	0.39	0.56	1.31
42	5	2	800	41.31	54.74	1.26	0.69	0.36	0.33	0.72	1.28
59	7	3	725	38.97	57.82	1.24	0.59	0.30	0.29	0.54	0.84
60	6	3	750	38.16	59.02	1.09	0.52	0.33	0.19	0.67	0.54
61	9	3	675	35.92	61.39	1.13	0.54	0.30	0.24	0.47	0.55
99	7	4	600	28.11	68.17	1.47	0.54	0.32	0.22	0.45	1.26
63	8	3	700	37.23	55.90	4.79	0.55	0.27	0.28	0.50	1.03
64	8	3	700	35.88	58.76	3.76	0.51	0.27	0.24	0.46	0.63
Average,	8	2.8	703	35.48	60.36	2.05	0.62	0.30	0.32	0.61	0.88
Max.,	12	4	800	41.31	68.17	4.79	0.87	0.36	0.56	0.92	2.21
Min.,	5	2	550	28.11	53.40	0.70	0.49	0.22	0.15	0.39	−0.33

MAPLE SIRUP. INOCULATED WITH RED YEAST

Calculated to a moisture-free basis

Sample number	Sucrose	Invert sugar	Total ash	Soluble ash	Insoluble ash	ALKALINITY		Malic acid value	Undetermined	Date
						Soluble ash	Insoluble ash			
	%	%	%	%	%			%	%	
14	95.30	3.01	1.30	0.46	0.84	33	144	0.88	—.49	4- 1-09
62	89.27	7.30	0.86	0.37	0.49	51	103	0.83	1.74	3-21-10
15	93.26	2.98	1.26	0.43	0.83	69	164	1.50	1.00	4- 1-09
114	96.31	1.01	0.71	0.49	0.22	61	52	0.56	1.41	3-30-11
18	91.65	3.12	1.03	0.39	0.64	49	112	1.03	3.17	4- 1-09
35	95.13	1.15	0.95	0.37	0.58	67	114	0.84	1.93	3- 7-10
42	93.27	2.14	1.18	0.61	0.57	58	122	1.23	2.18	3-14-10
59	94.74	2.03	0.97	0.49	0.48	65	99	0.88	1.38	3-21-10
60	95.44	1.76	0.85	0.53	0.32	58	91	1.08	0.87	"
61	95.80	1.77	0.84	0.46	0.38	64	81	0.73	0.86	"
99	94.82	2.05	0.75	0.44	0.31	57	63	0.67	1.71	3-26-11
63	89.06	7.63	0.88	0.43	0.45	63	91	0.80	1.63	3-21-10
64	91.64	5.86	0.79	0.42	0.37	54	82	0.72	0.99	"
Average,	93.54	3.18	0.96	0.47	0.49	58	101	0.91	1.41	
Max.,	96.31	7.63	1.30	0.61	0.84	69	164	1.50	3.17	
Min.,	89.06	1.01	0.71	0.37	0.22	33	52	0.56	−.49	

The moisture shows the usual variations, averaging 35.48%.

The sucrose averages 60.36, with a maximum of 68.17 and a minimum of 53.40%.

The invert sugar averages 2.05 and extremes range from 0.70 to 4.79%. This maximum is equivalent to 7.63% on a moisture free basis. The count secured on the plated sap previous to boiling seems to bear no direct relation to the amount of invert sugar. The count includes both bacterial and yeast colonies, however, so that the figures do not express the relative infection with yeasts. The variations in the invert sugar content may be assigned partly to differences in the individual strains employed and partly to the different degrees of infection secured. (See table 9, pages 392 to 395, Nos. 42, 63 and 64).

The total ash in Nos. 114 and 99 is below standard and the insoluble ash in No. 114 is also 0.01% low. The malic acid value is likewise a few points shy in No. 114. Both these samples were quite concentrated, their water contents being 30.60 and 28.11% respectively.

Group 7. *Inoculated with gray yeasts.* These organisms are closely associated with the red yeasts previously mentioned and, like them, are quite commonly found late in the sugar season. Their action is detrimental to a good flavored sirup, four out of the eight samples being graded 4 in flavor, corresponding to poor. In two cases the color was considerably darkened, being classed 10 and 11, corresponding to a No. 2 commercial grade. The invert sugar was increased in every case and a maximum percentage of 19.15% was found in No. 109.

TABLE 23. MAPLE SIRUP. INOCULATED WITH GRAY YEASTS

Sample number	Color	Flavor	Score	Moisture	Sucrose	Invert sugar	Total ash	Soluble ash	Insoluble ash	Malic acid value	Undetermined
				%	%	%	%	%	%	%	%
13	7	2	800	36.05	59.69	1.62	0.79	0.30	0.49	0.92	0.93
28	11	4	500	35.93	53.07	7.79	0.79	0.28	0.51	0.67	1.75
109	10	4	525	32.44	45.49	19.15	0.60	0.33	0.27	0.61	1.71
48	6	2[2]	775	38.49	57.75	1.44	0.63	0.35	0.28	0.51	1.18
52	5	2	850	38.37	59.68	0.75	0.56	0.30	0.26	0.51	0.13
53	6	3	750	41.50	53.90	2.32	0.49	0.32	0.17	0.60	1.19
110	6	4	625	30.94	67.05	1.20	0.46	0.30	0.16	0.46	—.11
115	7	4	600	31.04	65.61	1.05	0.50	0.35	0.15	0.43	1.37
Average,	7.3	3.2	678	35.60	57.78	4.42	0.60	0.32	0.28	0.59	1.01
Max.,	11	4	850	41.50	67.05	19.15	0.79	0.35	0.51	0.92	1.75
Min.,	5	2	500	30.94	45.49	0.75	0.46	0.28	0.15	0.43	—.11

MAPLE SIRUP. INOCULATED WITH GRAY YEAST

Calculated to a moisture-free basis

Sample number	Sucrose	Invert sugar	Total ash	Soluble ash	Insoluble ash	ALKALINITY: Soluble ash	ALKALINITY: Insoluble ash	Malic acid value	Undetermined	Date
	%	%	%	%	%			%	%	
13	93.34	2.53	1.24	0.47	0.77	56	141	1.44	1.45	4-10-09
28	82.83	12.15	1.23	0.44	0.79	62	151	1.04	2.75	3- 7-10
109	67.33	28.35	0.88	0.49	0.39	50	102	0.90	2.54	3-27-11
48	93.89	2.35	1.03	0.58	0.45	60	102	0.83	1.90	3-14-10
52	96.84	1.21	0.90	0.49	0.41	63	85	0.82	0.27	3-20-10
53	92.13	3.96	0.84	0.54	0.30	62	71	1.03	2.04	"
110	97.09	1.74	0.67	0.44	0.23	58	52	0.65	—.15	3-27-11
115	95.14	1.52	0.72	0.51	0.21	61	53	0.62	2.00	3-30-11
Average,	89.72	6.86	0.94	0.50	0.44	59	95	0.92	1.56	
Max.,	97.09	28.35	1.24	0.58	0.79	63	151	1.44	2.75	
Min.,	67.33	1.21	0.67	0.44	0.21	50	52	0.62	—.15	

The moisture content varied from 30.94 to 41.50 and averaged 35.60%.

The sucrose averaged 57.78% with extremes of 45.49 and 67.05%. The low figure which was obtained on No. 109 is due mainly to the high invert sugar content of 19.15%, equivalent on the moisture free material to 28.35%. This was the largest invert sugar percentage obtained with any of the samples examined. Reference to the field notes shows that the inoculating organism multiplied rapidly and was an acid producer, a condition which doubtless served to hasten the sucrose inversion.

The total ash on the moisture free basis shows a deficiency in two cases, Nos. 110 and 115. The insoluble ash is also low in No. 115. The malic acid value meets the standard in every case.

Group 8. *Inoculated with fluorescent bacteria.* These organisms occur more commonly in maple sap than do any of the others used in this work. They grow well at low temperatures and hence should be guarded against early in the season. They impair the flavor somewhat although not as seriously as several other groups. Nine of the 22 samples reported were graded as No. 2 in flavor, indicative of a good sirup, six as No. 3, a good medium sirup, while seven were classed as No. 4, corresponding to poor. The color was seriouly injured by this fluorescent group. They cause but little if any inversion of the sucrose and never produce the objectionable yeasty taste so characteristic of many contaminated sirups.

TABLE 24. MAPLE SIRUP. INOCULATED WITH FLUORESCENT BACTERIA

Sample number	Color	Flavor	Score	Moisture	Sucrose	Invert sugar	Total ash	Soluble ash	Insoluble ash	Malic acid value	Undetermined
				%	%	%	%	%	%	%	%
2	14	4	425	31.01	66.77	1.07	0.71	0.30	0.41	0.54	—.10
102	7	4	600	28.81	68.11	0.81	0.58	0.41	0.17	0.36	1.31
3	11	2	700	31.15	66.60	1.44	0.81	0.32	0.49	0.59	—.59
103	7	4	600	28.75	67.97	0.96	0.52	0.32	0.20	0.43	1.37
4	9	2	750	30.69	66.91	1.08	0.71	0.33	0.38	0.65	—.04
5	11	2	700	33.55	64.56	0.96	0.59	0.27	0.32	0.44	—.10
7	10	2	725	32.72	64.69	1.10	0.64	0.20	0.44	0.59	0.26
8	10	2	725	33.96	64.15	0.76	0.58	0.29	0.29	0.50	0.05
101	8	3	700	28.36	69.10	0.67	0.51	0.32	0.19	0.38	0.98
29	8	2[1]	750	37.31	59.50	0.24	0.67	0.24	0.43	0.54	1.74
30	8	2	775	32.91	64.90	0.60	0.71	0.30	0.41	0.63	0.25
44	9	3	675	38.72	57.01	0.86	0.64	0.25	0.39	0.70	2.07
100	7	3	725	27.76	69.59	0.67	0.57	0.39	0.18	0.40	1.01
69	9	3	675	37.17	58.91	1.00	0.76	0.24	0.52	0.79	1.37
70	11	4	500	36.72	59.15	1.56	0.80	0.34	0.46	0.70	1.07
71	14	4	425	37.87	58.20	0.36	*...	*...	*...	0.66	....
72	13	4	450	36.57	60.30	0.28	0.66	0.19	0.47	0.66	1.53
73	6	2	825	33.31	65.00	0.45	0.55	0.32	0.23	0.48	0.21
91	6	3	750	26.76	69.40	0.29	0.53	0.32	0.21	0.66	2.36
92	7	3	725	28.46	67.96	0.37	0.58	0.37	0.21	0.53	2.10
93	6	2	825	28.06	68.65	0.31	0.60	0.41	0.19	0.39	1.99
94	7	4	600	28.51	67.69	0.37	0.51	0.30	0.21	0.46	2.46
Average,	9	2.9	665	32.23	64.79	0.74	0.63	0.31	0.32	0.55	1.06
Max.,	14	4	825	38.72	69.59	1.56	0.81	0.41	0.52	0.79	2.46
Min.,	6	2	425	26.76	57.01	0.24	0.51	0.19	0.17	0.36	—.59

MAPLE SIRUP. INOCULATED WITH FLUORESCENT BACTERIA

Calculated to a moisture-free basis

Sample number	Sucrose	Invert sugar	Total ash	Soluble ash	Insoluble ash	ALKALINITY		Malic acid value	Undetermined	Date
						Soluble ash	Insoluble ash			
	%	%	%	%	%			%	%	
2	96.79	1.55	1.03	0.44	0.59	49	113	0.78	−0.15	3-24-09
102	95.67	1.14	0.82	0.58	0.24	64	58	0.50	1.87	3-26-11
3	96.73	2.09	1.18	0.47	0.71	52	113	0.86	−0.86	3-24-09
103	95.38	1.35	0.73	0.45	0.28	56	65	0.60	1.94	3-26-11
4	96.54	1.56	1.02	0.48	0.54	52	110	0.93	0.05	3-24-09
5	97.16	1.44	0.89	0.41	0.48	48	99	0.67	−0.16	3-24-09
7	96.16	1.64	0.95	0.30	0.65	54	104	0.88	0.37	3-27-09
8	97.14	1.15	0.88	0.44	0.44	42	82	0.75	0.08	"
101	96.45	0.94	0.71	0.44	0.27	60	56	0.53	1.37	3-26-11
29	94.91	0.38	1.07	0.39	0.68	61	131	0.87	2.77	3- 7-10
30	93.75	0.89	1.06	0.45	0.61	63	122	0.93	3.37	"
44	93.03	1.40	1.06	0.41	0.65	61	122	1.15	3.36	3-14-10
100	96.21	0.93	0.79	0.54	0.25	61	50	0.55	1.52	3-26-11
69	93.87	1.59	1.21	0.38	0.83	69	153	1.26	2.07	3-22-10
70	93.47	2.46	1.26	0.53	0.73	62	176	1.10	1.71	"
71	93.67	0.58	*...	*...	*...	*..	*..	1.06	....	"
72	95.07	0.44	1.03	0.29	0.74	64	150	1.03	2.43	"
73	97.47	0.68	0.83	0.48	0.35	70	66	0.71	0.31	"
91	94.76	0.40	0.72	0.44	0.28	58	58	0.90	3.22	3-22-11
92	95.00	0.52	0.81	0.51	0.30	57	76	0.74	2.93	"
93	95.43	0.43	0.83	0.56	0.27	70	54	0.54	2.77	"
94	94.68	0.52	0.72	0.41	0.31	57	45	0.64	3.44	"
Average,	95.42	1.09	0.93	0.46	0.47	59	95	0.82	1.74	
Max.,	97.47	2.46	1.26	0.58	0.83	70	176	1.26	3.44	
Min.,	93.03	0.38	0.71	0.29	0.24	48	45	0.50	—.86	

*Not determined through inadvertence.

The moisture ranged from 26.75 to 38.72, averaging 32.23%.

The sucrose variations, inasmuch as the invert sugar is low, follow the water content quite closely. The minimum found was 57.01, the maximum 69.59 and the average 64.79%.

The invert sugar variations of from 0.24 to 1.56, with the average of 0.74%, indicate quite conclusively that the inoculating organisms were not invert sugar formers.

The total ash as recorded in the moisture free basis table is below standard in Nos. 103, 101, 91 and 94. The insoluble ash meets all requirements in every case, while the malic acid value is low in four instances, Nos. 102, 101, 100 and 93.

GROUP 9. *Composites.* The saps from which this group of sirups was made were severally inoculated with from two to six organisms, hence the results are the product of a mixed infection. The object held in view in this procedure was to ascertain whether the presence in considerable numbers of two or more organisms would serve to stimulate or to reduce specific individual action. The flavor of all four samples was impaired, but the color was not as seriously affected. It is interesting to note that No. 19, inoculated with a mixture of fluorescent bacteria, yeasts and molds, contained a high invert sugar percentage and that the gray yeast used for inoculation purposes was the same organism employed in the inoculation of Nos. 13, 28 and 109 (table 23), in which the highest invert sugar contents obtained in this investigation were found.

TABLE 25. MAPLE SIRUP. COMPOSITES

Sample number	Color	Flavor	Score	Moisture	Sucrose	Invert sugar	Total ash	Soluble ash	Insoluble ash	Malic acid value	Undetermined
				%	%	%	%	%	%	%	%
19	9	3	675	32.46	63.00	2.76	0.69	0.23	0.46	0.86	0.23
116	8	4	575	29.89	67.04	0.48	0.60	0.45	0.15	0.46	1.53
118	7	4	600	29.30	67.66	0.69	0.59	0.41	0.18	0.41	1.35
119	7	2	800	28.75	68.59	0.58	0.46	0.30	0.16	0.42	1.20
Average,	7.7	3	663	30.10	66.57	1.13	0.59	0.35	0.24	0.53	1.08
Max.,	9	4	800	32.46	68.59	2.76	0.69	0.45	0.46	0.86	1.53
Min.,	7	2	575	28.75	63.00	0.48	0.46	0.23	0.15	0.41	0.23

MAPLE SIRUP. COMPOSITES

Calculated to a moisture-free basis

Sample number	Sucrose	Invert sugar	Total ash	Soluble ash	Insoluble ash	ALKALINITY: Soluble ash	ALKALINITY: Insoluble ash	Malic acid value	Undetermined	Date
	%	%	%	%	%			%	%	
19	93.28	4.09	1.02	0.34	0.68	50	127	1.27	0.34	4- 1-09
116	95.62	0.68	0.86	0.65	0.21	54	54	0.65	2.19	3-30-11
118	95.69	0.98	0.84	0.59	0.25	64	64	0.58	1.91	"
119	96.27	0.81	0.64	0.42	0.22	59	48	0.59	1.69	"
Average,	95.22	1.64	0.84	0.50	0.34	57	73	0.77	1.53	
Max.,	96.27	4.09	1.02	0.65	0.68	64	127	1.27	2.19	
Min.,	93.28	0.68	0.64	0.34	0.21	50	48	0.58	0.34	

The moisture content of the four samples in this group is low, indicating a greater density than is called for in standard sirup. It averaged 30.10% with extremes varying less than 4%.

The average sucrose content was 66.57%.

The invert sugar ranges well within the limits for average sirup, the high figure of 2.76% in No. 19, as has hitherto been pointed out, being occasioned by the activity of a gray yeast which in this and other instances exerted a pronounced inverting action on the sucrose.

The total ash is below standard in No. 119, the insoluble ash is low in Nos. 116 and 119, and the malic acid value just under the limit in Nos. 118 and 119.

GROUP 10. *Inoculated with Bacillus aceris* (new species). This organism causes a distinct type of stringiness in maple sap. It produces acid, inverts sucrose and forms gas. Fortunately for the sugar maker it does not appear to be very common in the sugar orchards. It exerts a most injurious effect on the flavor, two of the three samples inoculated scoring 4 and the other, 3. The color, while showing a depreciation from the control, does not appear to be affected to any great extent, for all three samples would have been commercially classed No. 1 in this particular. All the sirups had a cloudy appearance and the precipitated niter and other impurities settled out with difficulty. Previous to boiling the sap was quite stringy, but the resulting sirups were not especially viscid.

TABLE 26. MAPLE SIRUP. INOCULATED WITH *Bacillus acveris*

Sample number	Color	Flavor	Score	Moisture	Sucrose	Invert sugar	Total ash	Soluble ash	Insoluble ash	Malic acid value	Undetermined
				%	%	%	%	%	%	%	%
11	7	4	600	32.56	64.82	1.16	0.57	0.29	0.28	0.48	0.41
95	6	3	750	27.91	65.49	3.63	0.66	0.34	0.32	0.51	1.80
90	7	4	600	29.31	61.30	5.09	0.87	0.37	0.50	0.75	2.68
Average,	6.7	3.7	650	29.93	63.87	3.29	0.70	0.33	0.37	0.58	1.63
Max.,	7	4	750	32.56	65.49	5.09	0.87	0.37	0.50	0.75	2.68
Min.,	6	3	600	27.91	61.30	1.16	0.57	0.29	0.28	0.48	0.41

MAPLE SIRUP. INOCULATED WITH *Bacillus acveris*

Calculated to a moisture-free basis

Sample number	Sucrose	Invert sugar	Total ash	Soluble ash	Insoluble ash	ALKALINITY: Soluble ash	ALKALINITY: Insoluble ash	Malic acid value	Undetermined	Date
	%	%	%	%	%			%	%	
11	96.12	1.72	0.85	0.43	0.42	42	89	0.71	0.60	3-27-09
95	90.84	5.04	0.92	0.48	0.44	58	89	0.70	2.50	3-22-11
90	86.72	7.20	1.23	0.52	0.71	67	129	1.06	3.79	"
Average,	91.16	4.70	1.00	0.48	0.52	52	102	0.83	2.31	
Max.,	96.12	7.20	1.23	0.52	0.71	67	129	1.06	3.79	
Min.,	86.72	1.72	0.85	0.43	0.42	42	89	0.70	0.60	

The moisture content of these sirups varied from 27.91 to 32.56 and averaged 29.93%.

The sucrose showed relatively small variations, ranging from 61.30 to 65.49 and averaging 63.87%.

The invert sugars tend to run high, an average of 3.29% being found with extremes of 1.16 to 5.09%. The fact that

all the saps were decidedly acid after inoculation with this organism, would serve to explain the high invert sugar content.

The ash and malic acid value figures are all well above the Vermont standard.

GROUP 11. *Last run, sweet.* These samples were secured late in each of three sugar seasons, 3 in 1909, 1 in 1910 and 5 in 1911. The trees were freshly tapped and clean spouts and pails used. The sap appeared bright and clear and, with one exception, was boiled down immediately after collection. Hence the conditions under which these saps were secured may be deemed to be beyond criticism.

The three samples taken in 1909 displayed exceptionally fine flavor and color, all grading 1 in flavor and 3, 7, and 5 in color and scoring 950, 850 and 900 respectively (pages 357-358). The single sample secured in 1910 and the five obtained in 1911, however, while being extremely light colored, had a pronounced "buddy" flavor, which renders a sirup particularly obnoxious for food purposes. As maple sirup is usually purchased for its flavor rather than for its sweetening properties, sirup which is thus affected is desired by neither manufacturer, dealer, nor the purchasing public.

"Buddy" sirup is secured only when a freeze occurs after the true sugar season has ended and a few warm days have started the development of the leaf buds. Sap will run on suitable days following such freezes, and if the trees are retapped and clean utensils used, the grade of goods now being considered will be secured. Should the old tap-holes, spouts and buckets be used, the material will possess a mixture of "buddy" and other objectionable flavors, due to the contamination of the spouts and pails[1]. This latter condition will be again referred to in table 32 on page 455 and in the appended discussion.

[1]In this connection note the statements made on pages 379 and 417 concerning "buddy" flavor and its probable origin, as well as the statement immediately succeeding table 27 on page 446.

TABLE 27. MAPLE SIRUP. LAST RUN SWEET

Sample number	Color	Flavor	Score	Moisture	Sucrose	Invert sugar	Total ash	Soluble ash	Insoluble ash	Malic acid value	Undetermined
				%	%	%	%	%	%	%	%
21	3	1	950	34.31	62.53	0.54	0.54	0.33	0.21	0.73	1.35
24	7	1	850	29.41	67.75	0.43	0.50	0.33	0.17	0.71	1.20
26	5	1	900	32.67	64.92	0.34	0.54	0.31	0.23	0.67	0.86
87	4	5	525	38.12	60.19	0.16	0.49	0.26	0.23	0.61	0.43
122	5	5	500	32.55	64.61	0.45	0.49	0.30	0.19	0.52	1.38
124	5	5	500	29.35	69.62	0.42	0.52	0.36	0.16	0.43	0.34
126	6	5	475	32.71	63.56	0.21	*...	*...	*...	0.64	...
128	4	5	525	31.81	64.23	0.21	0.40	0.20	0.20	0.56	2.79
129	5	5	500	28.81	67.87	0.08	0.54	0.36	0.18	0.44	2.26
Average,	5	3.7	636	32.20	65.03	0.32	0.50	0.30	0.20	0.58	1.37
Max.,	7	5	950	38.12	69.62	0.54	0.54	0.36	0.23	0.73	2.79
Min.,	3	1	475	28.81	60.19	0.08	0.40	0.20	0.16	0.43	.34

MAPLE SIRUP. LAST RUN SWEET

Calculated to a moisture-free basis

Sample number	Sucrose	Invert sugar	Total ash	Soluble ash	Insoluble ash	ALKALINITY: Soluble ash	ALKALINITY: Insoluble ash	Malic acid value	Undetermined	Date
	%	%	%	%	%			%	%	
21	95.19	0.82	0.83	0.50	0.33	58	91	1.11	2.05	4-12-09
24	96.02	0.61	0.71	0.47	0.24	54	54	1.01	1.65	"
26	96.42	0.51	0.80	0.46	0.34	54	62	1.00	1.27	4-16-09
87	97.27	0.26	0.79	0.41	0.38	68	145	0.98	0.52	4-23-10
122	95.80	0.67	0.73	0.45	0.28	68	59	0.76	2.04	4-21-11
124	98.54	0.59	0.74	0.52	0.22	56	47	0.61	0.48	4-24-11
126	94.46	0.31	*...	*...	*...	*..	*..	0.95	....	"
128	94.21	0.31	0.59	0.29	0.30	48	56	0.82	4.07	4-27-11
129	95.37	0.12	0.75	0.51	0.24	59	45	0.61	3.15	4-28-11
Average,	95.92	0.47	0.74	0.45	0.29	58	70	0.87	2.00	
Max.,	98.54	0.82	0.83	0.52	0.38	68	145	1.11	4.07	
Min.,	94.21	0.12	0.59	0.29	0.22	48	45	0.61	.48	

*Not determined through inadvertence.

The moisture content of these sirups averaged 32.20, the maximum being 38.12 and the minimum 28.81%.

The sucrose averaged 65.03 with extremes of 60.19 and 69.62%.

The invert sugar was exceptionally small in amount, averaging only 0.32% and varying between 0.08 and 0.54%. This in itself is proof that care was taken in securing the sap and that no time was lost before boiling. The low invert sugar, together with the exceptional light color, also leads to the inference that the objectionable flavor was not caused by external contamination, but was due to some change or impurity in the sap before it left the tree, occasioned by changes occurring inside the tree, incident to the renewal of the yearly spring functions.

The total ash content runs low in 5 out of 8 samples, the average on a moisture free basis being 0.74, the maximum 0.83, and the minimum, 0.59%. The insoluble ash is low in but one instance, No. 124 being 0.01% deficient. The malic acid value meets requirements in every case.

GROUP 12. *Inoculated with fluorescent bacteria and with spore-bearers.* The saps used in securing these samples were inoculated with various strains of bacteria, mainly of the fluorescent type. Through inadvertence, spore-bearing organisms of the subtilis type commonly found in soil gained entrance[1]. The results were strikingly influenced by the presence and action of these spore-bearing organisms. The flavor was seriously injured, 4 out of 7 samples grading 4 (poor) and the rest of them 3 (medium). The color was darkened several shades and showed a large depreciation as compared with the controls, although none were classed below a No. 2, as commercially rated. By comparison with the results obtained with fluorescent organisms alone, it appears that the color injury should be attributed largely to the introduced species, while the intruder is doubtless chiefly responsible for the ill flavor and the high invert sugar content.

[1] See statement concerning this matter, page 377.

TABLE 28. MAPLE SIRUP. INOCULATED WITH FLUORESCENT BACTERIA AND OTHER ORGANISMS AND WITH SPORE-BEARERS

Sample number	Color	Flavor	Score	Moisture	Sucrose	Invert sugar	Total ash	Soluble ash	Insoluble ash	Malic acid value	Undetermined
				%	%	%	%	%	%	%	%
75	9	3	675	40.32	50.57	6.43	0.78	0.26	0.52	0.73	1.17
76	10	3	650	38.02	53.94	5.54	0.80	0.34	0.46	0.76	0.94
77	8	3	700	39.67	53.17	4.97	0.79	0.34	0.45	0.80	0.60
78	8	4	575	39.98	50.33	6.29	0.74	0.24	0.50	0.71	1.95
79	7	4	600	47.60	41.28	7.99	0.67	0.19	0.48	0.74	1.72
80	10	4	525	45.03	43.54	8.81	0.72	0.24	0.48	0.73	1.17
81	7	4	600	38.72	54.68	4.60	0.69	0.34	0.35	0.66	0.65
Average,	8.4	3.6	618	41.33	49.64	6.38	0.74	0.27	0.47	0.73	1.18
Max.,	10	4	700	47.60	54.68	8.81	0.80	0.34	0.52	0.80	1.95
Min.,	7	3	525	38.02	41.28	4.60	0.67	0.19	0.35	0.66	0.60

MAPLE SIRUP. INOCULATED WITH FLUORESCENT BACTERIA AND OTHER ORGANISMS AND WITH SPORE-BEARERS

Calculated to a moisture-free basis

Sample number	Sucrose	Invert sugar	Total ash	Soluble ash	Insoluble ash	ALKALINITY: Soluble ash	ALKALINITY: Insoluble ash	Malic acid value	Undetermined	Date
	%	%	%	%	%			%	%	
75	84.74	10.77	1.30	0.43	0.87	71	118	1.22	1.97	3-24-10
76	87.03	8.94	1.29	0.54	0.75	59	70	1.23	1.51	"
77	88.13	8.24	1.31	0.56	0.75	62	86	1.32	1.00	"
78	83.86	10.48	1.24	0.40	0.84	61	169	1.18	3.24	"
79	78.78	15.25	1.28	0.36	0.92	64	184	1.42	3.27	"
80	79.20	16.03	1.30	0.43	0.87	61	175	1.32	2.15	"
81	89.23	7.51	1.12	0.56	0.56	62	50	1.07	1.07	"
Average,	84.42	11.03	1.26	0.46	0.80	63	122	1.25	2.04	
Max.,	89.23	16.03	1.31	0.56	0.92	71	184	1.42	3.27	
Min.,	78.78	7.51	1.12	0.36	0.56	59	50	1.07	1.00	

The entire lot of 7 samples were a light weight sirup, the moisture content running from 47.60 to 38.02 and averaging 41.33%. The sucrose is low owing to the high water and invert sugar content, averaging but 49.64, with extremes of 41.28 and 54.68%. The invert sugar runs uniformly high and without the extreme variations noted in some of the previous groups. The extremes are 4.60 and 8.81 with an average of 6.38%, which, on the moisture free basis, is equivalent to 11.03%. The ash and malic acid value data are well above standard.

GROUP 13. *Inoculated with green molds.* The organisms here used are the ordinary green molds frequently seen on stale bread. While of common occurrence, they do not grow vigorously at low temperatures, hence they are of minor importance so far as maple sap is concerned; but probably no other single group of organisms does so much damage to sirup after its manufacture. When introduced into the sap, they seriously impaired both the flavor and color of the resulting sirup, 3 out of the 4 samples grading 4 in flavor, corresponding to poor quality, while the color showed a depreciation of over 4 points from the control. The content of invert sugar was also noticeably increased.

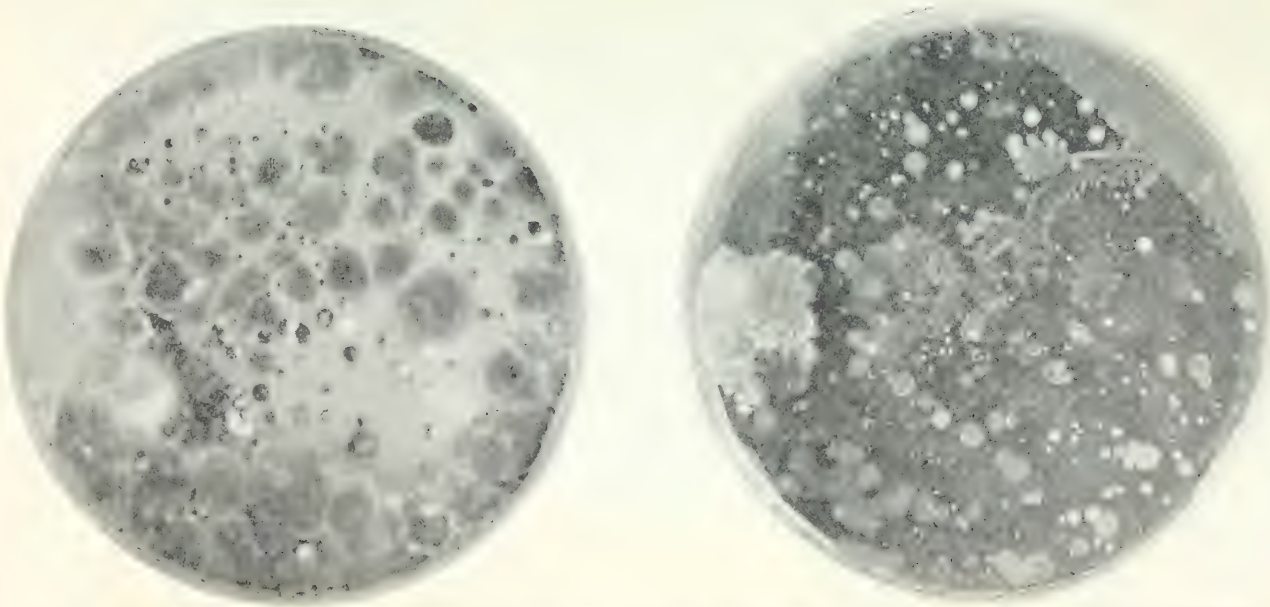

PLATE VII.—Colonies developing on nutrient agar and on synthetic agar, respectively, from one-two thousandth of a cubic centimeter of maple sap No. 67 received in wooden buckets. (See pages 343, 373.)

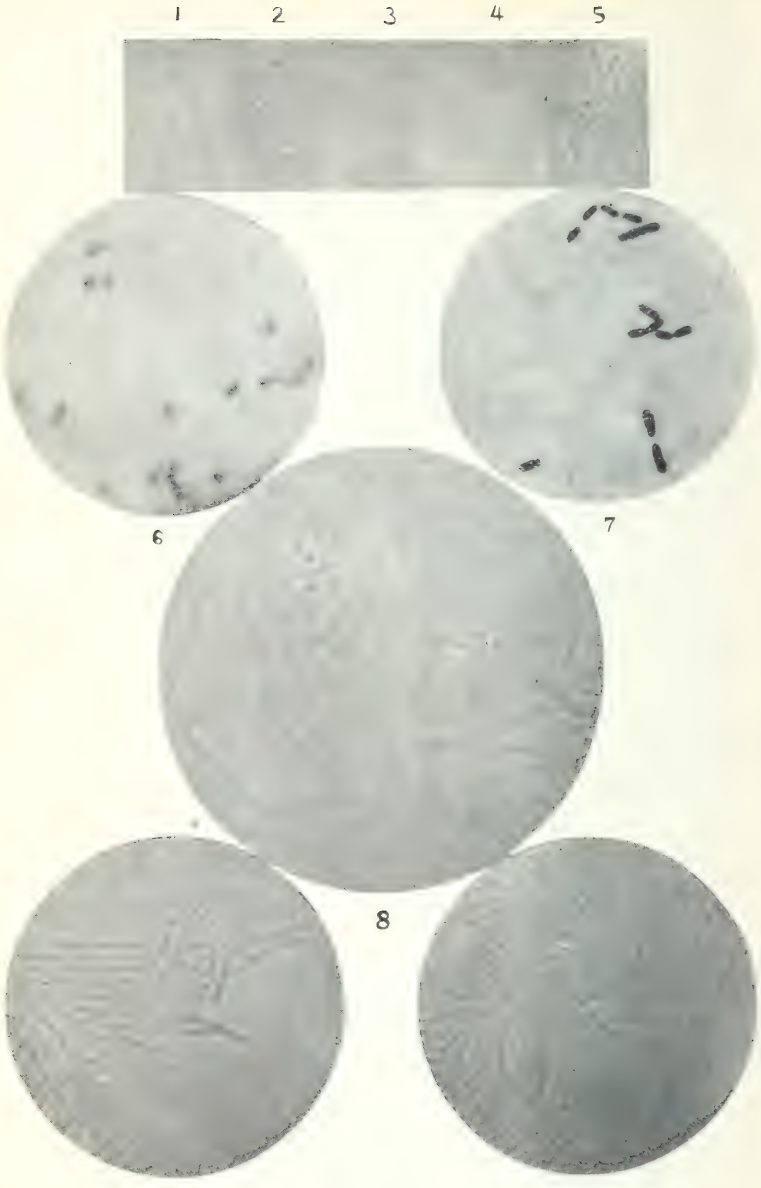

PLATE VIII.—*Bacillus aceris.* Figures 1-5. Successive photographs of living organisms during fission. Figure 6. Flagella preparations. Löwet's stain. Figure 7. Stained preparation, gentian violet. Figure 8. Living organisms showing chains in motion. Figures 9 and 10. Living organisms on agar, showing orientation. (See pages 480-482.) ×1350.

TABLE 29. MAPLE SIRUP. INOCULATED WITH GREEN MOLDS

Sample number	Color	Flavor	Score	Moisture	Sucrose	Invert sugar	Total ash	Soluble ash	Insoluble ash	Malic acid value	Undetermined
				%	%	%	%	%	%	%	%
16	10	4	525	32.86	64.27	1.75	0.72	0.24	0.48	0.49	-0.09
17	10	2	725	34.08	61.15	2.29	0.72	0.20	0.52	0.95	0.81
111	7	4	600	28.29	66.91	3.15	0.55	0.38	0.17	0.48	0.62
117	9	4	550	30.70	63.12	2.58	0.51	0.35	0.16	0.42	2.67
Average,	9	3.5	600	31.48	63.86	2.44	0.63	0.30	0.33	0.58	1.01
Max.,	10	4	725	34.08	66.91	3.15	0.72	0.38	0.52	0.95	2.67
Min.,	7	2	525	28.29	61.15	1.75	0.51	0.20	0.16	0.42	-0.09

MAPLE SIRUP. INOCULATED WITH GREEN MOLDS

Calculated to a moisture-free basis

Sample number	Sucrose	Invert sugar	Total ash	Soluble ash	Insoluble ash	ALKALINITY		Malic acid value	Undetermined	Date
						Soluble ash	Insoluble ash			
	%	%	%	%	%			%	%	
16	95.73	2.61	1.07	0.36	0.71	39	140	0.72	-0.13	4- 1-09
17	92.76	3.47	1.09	0.30	0.79	46	161	1.44	1.24	"
111	93.31	4.39	0.77	0.53	0.24	61	57	0.67	0.86	3-27-11
117	91.09	3.72	0.74	0.51	0.23	64	52	0.61	3.84	3-30-11
Average,	93.21	3.56	0.92	0.44	0.48	52	103	0.86	1.45	
Max.,	95.73	4.39	1.09	0.53	0.79	64	161	1.44	3.84	
Min.,	91.09	2.61	0.74	0.30	0.23	39	52	0.61	-0.13	

The moisture shows less variation than usual, the extremes being 34.08 and 28.29 and the average 31.48%. The sucrose averages 63.86, with a minimum of 61.15, and a maximum of 66.91%. The invert sugar figures run fairly high, the extremes being 1.75 and 3.15 and the average 2.44%. Three green mold organisms were used in the inoculation of these samples and

they all appear to exert a marked inverting action on sucrose. The total and insoluble ashes together with malic acid value are above standard in all cases.

GROUP 14. *Tin vs. wooden buckets.* The sap from 6 trees was used in this phase of the work. All tap-holes, spouts and utensils were made strictly clean and the procedures were identical in both cases. The sap was concentrated as soon as collected. That obtained in the tin buckets ran 2 days earlier than that gathered in the wooden buckets, but, as a check, in order to be certain that the two days' intermission exercised no influence on the results, bacterial counts were made on sap obtained in tin buckets from the same trees and spouts after the close of the experiment. It was practically free from bacteria of any kind; (see page 343). The grade of sirup produced from the tin buckets (No. 66) was of the highest quality, ranking 1 in flavor and 3 in color, and scoring 950. That secured from the wooden buckets (No. 67), in marked contrast to that made when tin buckets were employed, ranked only 4 in flavor, 6 in color and scored but a total of 550 points out of a possible 975. Furthermore its invert sugar content was high, there being seventeen times as much present in the sirup secured in the wooden buckets as was found in that made when tin buckets were used. This is doubtless to be attributed to the organisms already existing in the wooden buckets since, although thoroughly cleansed, they were not new and were painted on the outside only.

Both sirups were light in weight containing respectively 37.78 and 38.33 percents of water.

The sucrose is considerably higher in No. 66 than in No. 67, owing to its low invert sugar content.

The standards for ash and malic acid value are fully met by both samples.

TABLE 30. MAPLE SIRUP. TIN VS. WOODEN BUCKETS

Sample number	Color	Flavor	Score	Moisture	Sucrose	Invert sugar	Total ash	Soluble ash	Insoluble ash	Malic acid value	Undetermined
				%	%	%	%	%	%	%	%
66	3	1	950	37.78	60.12	0.25	0.49	0.31	0.18	0.52	0.84
67	9	4	550	38.33	54.42	4.26	0.62	0.34	0.28	0.73	1.64

MAPLE SIRUP. TIN VS. WOODEN BUCKETS

Calculated to a moisture-free basis

Sample number	Sucrose	Invert sugar	Total ash	Soluble ash	Insoluble ash	ALKALINITY		Malic acid value	Undetermined	Date
						Soluble ash	Insoluble ash			
	%	%	%	%	%			%	%	
66	96.62	0.41	0.78	0.49	0.29	65	59	0.84	1.35	3-22-10
67	88.25	6.91	1.01	0.55	0.46	63	103	1.19	2.64	3-24-10

GROUP 15. *Inoculated with pink yeast; burned control.* These two samples, while grouped together, bear no relation to each other. The pink yeasts are not as common as are the red and gray yeasts previously mentioned, but, like them, are encountered late in the season. The effect on sirup made from sap inoculated with pink yeast is shown in No. 98, which may be considered a typical yeast sirup. Both flavor and color were impaired, particularly the former. The sirup ranked poor in quality and, while grading commercially as No. 2, showed a color depreciation of 6 points. This organism exerted a marked inverting action on the sucrose, but the other results, including those for ash and malic acid value, are normal in spite of the increased concentration, indicated by the low moisture content of 29.26%.

The burned sample, No. 74, listed in this table, was a control on the fluorescent and spore-bearer series shown in table 12. It was slightly burned, so that the flavor and color were seriously affected. The burning had but little if any effect on the invert sugar percentage, as only 0.70% calculated to the moisture free basis was found.

TABLE 31. MAPLE SIRUP. INOCULATED WITH PINK YEAST; BURNED CONTROL

Sample number	Color	Flavor	Score	Moisture	Sucrose	Invert sugar	Total ash	Soluble ash	Insoluble ash	Malic acid value	Undetermined
				%	%	%	%	%	%	%	%
98	9	4	550	29.26	61.33	6.15	0.59	0.37	0.22	0.50	2.17
74	9	4	550	41.40	56.47	0.41	0.73	0.24	0.49	0.77	0.22

MAPLE SIRUP. INOCULATED WITH PINK YEAST; BURNED CONTROL.

Calculated to a moisture-free basis

Sample number	Sucrose	Invert sugar	Total ash	Soluble ash	Insoluble ash	ALKALINITY		Malic acid value	Undetermined	Date
						Soluble ash	Insoluble ash			
	%	%	%	%	%			%	%	
98	86.70	8.69	0.83	0.52	0.31	62	74	0.71	3.07	3-26-11
74	96.36	0.70	1.24	0.41	0.83	51	99	1.31	0.39	3-24-10

GROUP 16. *Last run sour.* The sirups thus listed were made from sap which was collected late in the season, and which was cloudy as it dripped from the spout. The tap-holes, spouts and buckets were contaminated. In every case but one the sap was evaporated on the same day it ran.

Referring to the column headed flavor in the following table, it will be noted that the three samples, Nos. 22, 23 and 25, secured late in the season of 1909, graded 3 in flavor, corresponding to medium quality, and 8, 14 and 14 respectively in color, indicating the second and third grades. No "buddy" sirup was obtained in 1909. The sirups secured in 1910 and 1911 graded 5 or 6 in flavor indicating buddiness or worse than buddiness. The color in all but Nos. 121 and 125 was seriously affected, grading from 11 to 15.

TABLE 32. MAPLE SIRUP. LAST RUN SOUR

Sample number	Color	Flavor	Score	Moisture	Sucrose	Invert sugar	Total ash	Soluble ash	Insoluble ash	Malic acid value	Undetermined
				%	%	%	%	%	%	%	%
22	8	3	700	34.45	62.45	1.81	0.64	0.42	0.22	0.87	−0.22
23	14	3	550	34.73	60.41	2.32	0.74	0.40	0.34	0.58	1.22
25	14	3	550	38.68	57.67	1.92	0.86	0.26	0.60	1.15	−0.28
88	11	6	250	35.57	60.93	1.15	0.80	0.35	0.45	0.83	0.72
121	7	5	450	35.85	60.26	0.22	0.59	0.25	0.35	0.69	0.59
123	11	6	250	34.01	62.82	0.42	0.54	0.28	0.26	0.55	1.66
125	7	5	450	35.67	60.39	0.34	0.53	0.27	0.26	0.58	2.49
127	15	6	150	33.36	58.25	3.06	0.80	0.32	0.48	0.85	3.68
130	12	6	225	33.41	62.50	0.89	0.68	0.34	0.34	0.65	1.87
Av'ge,	11	4.8	397	35.08	60.63	1.35	0.68	0.31	0.37	0.75	1.51
Max.,	15	6	700	38.68	62.82	3.06	0.86	0.42	0.60	1.15	3.68
Min.,	7	3	150	33.36	57.67	0.22	0.53	0.25	0.22	0.55	0.28

MAPLE SIRUP. LAST RUN SOUR

Calculated to a moisture-free basis

Sample number	Sucrose	Invert sugar	Total ash	Soluble ash	Insoluble ash	ALKALINITY: Soluble ash	ALKALINITY: Insoluble ash	Malic acid value	Undetermined	Date
	%	%	%	%	%			%	%	
22	95.27	2.76	0.98	0.64	0.34	85	76	1.33	0.34	4-12-09
23	92.56	3.55	1.13	0.61	0.52	58	172	0.90	1.86	"
25	94.05	3.13	1.40	0.42	0.98	98	173	1.87	−0.45	4-16-09
88	94.56	1.79	1.24	0.54	0.70	52	173	1.29	1.12	4- 3-10
121	93.94	0.34	0.91	0.38	0.53	59	98	1.08	3.73	4-21-11
123	95.33	0.64	0.83	0.43	0.40	56	73	0.84	2.36	4-24-11
125	93.88	0.53	0.82	0.41	0.41	53	94	0.90	3.87	"
127	87.41	4.59	1.20	0.49	0.71	59	144	1.28	5.52	4-27-11
130	93.86	1.34	1.03	0.52	0.57	71	99	0.98	2.79	4-28-11
Average,	93.41	2.07	1.05	0.48	0.57	64	122	1.16	2.31	
Max.,	95.33	4.59	1.40	0.64	0.98	98	173	1.87	5.52	
Min.,	87.41	0.34	0.82	0.38	0.34	52	73	0.84	0.45	

The moisture content of these sirups showed less variation than has been noted in many of the groups. The average was 35.08, with extremes of 38.68 and 33.36%.

The sucrose averaged 60.63, with extremes of 57.67 and 62.82%.

The invert sugar averaged 1.35%. Nos. 121, 123 and 125 contained but small amounts, which serves to show that in these cases the infecting organism did not act as an invert sugar former. The remaining samples, however, show quite a marked increase, the invert sugar content of No. 127 being 3.06%.

The total and insoluble ash and malic acid value are well above the standard in every case.

GROUP 17. *Sour sap, kept.* The three samples thus listed were secured in 1910 just previous to the buddy sample No. 88 mentioned in table 32. They represent a composite of the small runs during several days toward the close of the season. Tapholes, spouts and pails were contaminated through the ordinary natural sources, as was indicated by the cloudy appearance of the running sap. The flavor in each case was a poor medium. The color of No. 84 was darker than that of any other sample secured, with two exceptions grading 20, the extreme limit on the colorimetric scheme. Nos. 85 and 86 were even darker than No. 84 and defied grading. An attempt was made during the boiling process to clarify No. 86 by means of the usual white of egg treatment which, in this case at least, was ineffective.

TABLE 33. MAPLE SIRUP. FROM SOUR SAP KEPT

Sample number	Color	Flavor	Score	Moisture	Sucrose	Invert sugar	Total ash	Soluble ash	Insoluble ash	Malic acid value	Undetermined
				%	%	%	%	%	%	%	%
84	20	3¹	350	33.67	60.45	1.88	0.99	0.34	0.65	0.65	2.36
85	20+	3¹	325	36.67	59.96	0.99	0.89	0.23	0.66	0.73	0.76
86	20+	3¹	325	34.72	60.85	0.81	0.91	0.27	0.64	0.82	1.89
Av'ge,	20	3¹	333	35.02	60.42	1.23	0.93	0.28	0.65	0.73	1.67
Max.,	20+		350	36.67	60.85	1.88	0.99	0.34	0.66	0.82	2.36
Min.,	20		325	33.67	59.96	0.81	0.89	0.23	0.64	0.65	0.76

MAPLE SIRUP. FROM SOUR SAP KEPT

Calculated to a moisture-free basis

Sample number	Sucrose	Invert sugar	Total ash	Soluble ash	Insoluble ash	ALKALINITY		Malic acid value	Undetermined	Date
						Soluble ash	Insoluble ash			
84	91.14	2.83	1.50	0.51	0.99	75	122	0.98	3.55	4- 2-10
85	94.68	1.57	1.41	0.37	1.04	63	130	1.15	1.19	"
86	93.21	1.23	1.40	0.42	0.99	65	137	1.26	2.90	"
Average,	93.00	1.88	1.44	0.43	1.01	68	130	1.13	2.55	
Max.,	94.68	2.83	1.50	0.51	1.04	75	137	1.26	3.55	
Min.,	91.14	1.23	1.40	0.37	0.99	63	122	0.98	1.19	

The moisture content of the three sirups varied from 36.67 to 33.67, averaging 35.02%.

The sucrose content was quite uniform and averaged 60.42%.

The invert sugar percentages were 1.88, 0.99 and 0.81, averaging 1.23%. These figures are low enough to indicate that, although the contaminating organisms were very abundant, bacterial counts showing over 11,000,000 per cc., they were relatively inactive as producers of invert sugar from sucrose. As has been stated the color of the sirup was most seriously affected.

Both the total and insoluble ash contents as well as the malic acid values were above standard.

SUMMARY OF AVERAGES SECURED ON THE SUNDRY GROUPS DISCUSSED

Table 34 displays the averages of the individual analyses of the several sirups examined, in the order previously discussed, together with the average for the 128 samples. The differences in physical characteristics and chemical composition are indicated in the original material and the moisture-free basis portions of the table.

The color averages 7.5, corresponding closely to first grade. The darkest color was obtained in the samples located in the "sour sap, kept" group. This group also showed the highest depreciation from the control as regards color. The lightest color was secured in samples grouped under the captions "tin buckets" and "control."

The flavor averages 2.9, corresponding to a quality just below medium. The finest flavor was obtained in samples grouped under the term "tin buckets" and "control" rating as 1 and 1.4 respectively, the poorest sample in this respect was located in the group denominated "last run, sour," which included several buddy sirups. Excluding the samples rating 5 and 6 in flavor (buddy) from the color and flavor averages, the average color and flavor figures thus revised for the remaining 116 samples are 7.5 and 2.6 respectively, equivalent to an average score of 719.

TABLE 34. AVERAGE ANALYSES OF THE MAPLE SIRUP GROUPS

Group number	Character of organism	Color	Flavor	Score	Moisture	Sucrose	Invert sugar	Total ash	Soluble ash	Insoluble ash	Malic acid value	Undetermined
					%	%	%	%	%	%	%	%
1	Control	3.8	1.4	911	34.06	63.51	0.39	0.52	0.28	0.24	0.57	0.95
2	Incubator control	5.4	2.0	827	36.78	60.41	0.65	0.57	0.29	0.28	0.55	1.04
3	Non-fluorescent	5.5	2.5	800	34.70	62.36	0.99	0.69	0.27	0.42	0.57	0.69
4	Pink cocci	5.7	2.6	775	37.15	59.05	1.54	0.54	0.28	0.26	0.50	1.22
5	Failures	7.0	2.3	772	34.99	62.48	0.33	0.65	0.29	0.36	0.52	1.03
6	Red yeasts	8.0	2.8	703	35.48	60.36	2.05	0.62	0.30	0.32	0.61	0.88
7	Gray yeasts	7.3	3.2	678	35.60	57.78	4.42	0.60	0.32	0.28	0.59	1.01
8	Fluorescent	9.0	2.9	665	32.23	64.79	0.74	0.63	0.31	0.32	0.55	1.06
9	Composite	7.7	3.0	663	30.10	66.57	1.13	0.59	0.35	0.24	0.53	1.08
10	*Bacillus aceris*	6.7	3.7	650	29.93	63.87	3.29	0.70	0.33	0.37	0.58	1.63
11	Last run, sweet	5.0	3.7	636	32.20	65.03	0.32	0.50	0.30	0.20	0.58	1.37
12	Fluor. and spore-bearers	8.4	3.6	618	41.33	49.64	6.38	0.74	0.27	0.47	0.73	1.18
13	Green molds	9.0	3.5	600	31.48	63.86	2.44	0.63	0.30	0.33	0.58	1.01
14	Tin buckets	3.0	1.0	950	37.78	60.12	0.25	0.49	0.31	0.18	0.52	0.84
14	Wooden buckets	9.0	4.0	550	38.53	54.42	4.26	0.62	0.34	0.28	0.73	1.64
15	Pink yeasts	9.0	4.0	550	29.26	61.33	6.15	0.59	0.37	0.22	0.50	2.17
15	Burned control	9.0	4.0	550	41.40	56.47	0.41	0.73	0.24	0.49	0.77	0.22
16	Last run, sour	11.0	4.8	397	35.08	60.63	1.35	0.68	0.31	0.37	0.75	1.51
17	Sour sap, kept	20.0	3.3	333	35.02	60.42	1.23	0.93	0.28	0.65	0.73	1.67
	Average	7.5	2.9	689	34.63	61.44	1.60	0.61	0.29	0.32	0.59	1.13

AVERAGE ANALYSES OF THE MAPLE SIRUP GROUPS
Calculated to a moisture-free basis

Group number	Character of organism	Sucrose	Invert sugar	Total ash	Soluble ash	Insoluble ash	Alkalinity: Soluble ash	Alkalinity: Insoluble ash	Malic acid value	Undetermined	Number of samples
		%	%	%	%	%			%	%	
1	Control	96.32	0.60	0.77	0.41	0.36	51	77	0.86	1.45	11
2	Incubator control	95.55	1.04	0.90	0.46	0.44	61	92	0.87	1.64	13
3	Non-fluorescent	95.50	1.52	1.06	0.42	0.64	46	132	0.87	1.05	4
4	Pink cocci	93.94	2.45	0.86	0.45	0.41	61	83	0.79	1.96	6
5	Failures	96.13	0.51	1.00	0.45	0.55	59	98	0.80	1.56	8
6	Red yeasts	93.54	3.18	0.96	0.47	0.49	58	101	0.91	1.41	13
7	Gray yeasts	89.72	6.86	0.94	0.50	0.44	59	95	0.92	1.56	8
8	Fluorescent	95.42	1.09	0.93	0.46	0.47	59	95	0.82	1.74	22
9	Composite	95.22	1.64	0.84	0.50	0.34	57	73	0.77	1.53	4
10	Bacillus aceris	91.16	4.70	1.00	0.48	0.52	52	102	0.83	2.31	3
11	Last run, sweet	95.92	0.47	0.74	0.45	0.29	58	70	0.87	2.00	9
12	Fluor. bac. & spore bearers	84.42	11.03	1.26	0.46	0.80	63	122	1.25	2.04	7
13	Green molds	93.21	3.56	0.92	0.44	0.48	52	103	0.86	1.45	4
14	Tin buckets	96.62	0.41	0.78	0.49	0.29	65	59	0.84	1.35	1
14	Wooden buckets	88.25	6.91	1.01	0.55	0.46	36	103	1.19	2.64	1
15	Pink yeasts	86.70	8.69	0.83	0.52	0.31	62	74	0.71	3.07	1
15	Burned, control	96.36	0.70	1.24	0.41	0.83	51	99	1.31	0.39	1
16	Last run, sour	93.41	2.07	1.05	0.48	0.57	64	122	1.16	2.31	9
17	Sour sap, kept	93.00	1.88	1.44	0.43	1.01	68	130	1.13	2.55	3
	Average	93.99	2.45	0.93	0.44	0.49	59	97	0.90	1.73	
	Maximum	96.62	11.03	1.44	0.55	1.01	68	132	1.31	3.07	
	Minimum	84.42	0.41	0.74	0.41	0.29	36	59	0.71	0.39	

The average moisture content for the entire 128 samples is 34.63%. This is practically the moisture percentage of a standard eleven-pound to the gallon sirup.

The average sucrose figures 61.44%, and the invert sugar percentage, 1.60%, agree quite closely with the averages, 62.04% and 1.49%, secured by Bryan in an examination of 395 samples from all parts of the United States where maple products are made.

The average total ash figure on a moisture free basis is 0.93%, and extremes are 0.74 and 1.44%. The minimum figures occur in the "last run, sour" group and include considerable number of buddy sap samples.

The average insoluble ash percentage is well over the standard in every group, the minimum being 0.29, the maximum 1.01, and the average 0.59%.

The malic acid value is likewise above the standard limit, with a minimum of 0.71, a maximum of 1.21, and an average of 0.90%.

The grand average for the 128 samples secured in the three sugar seasons is in every particular typical of pure maple sirup, and would of course more nearly represent the output of that particular sugar orchard than would any single sample or minor group of samples.

INVERT SUGAR CONTENT OF MAPLE SIRUP

The invert sugar present in the sirups obtained in this investigation shows extremes, calculated to a moisture-free basis, of 0.12 and 28.35%. Invert sugar results from the hydrolysis or inversion of sucrose, caused by yeasts, molds, bacteria, acids, etc. Thirty-two sirups, or a quarter part of the entire number of samples, carried less than 0.60% of invert sugar on a moisture-free basis, while 57 samples, or 45%, contained less than 1%. Hence it seems fair to conclude that an invert sugar content in maple sirup of much more than 1% can only be due to careless methods in handling or to delay in boiling the sap, or to the subsequent fermentation of the finished product.

The average invert sugar in the sirups examined, together with the maximum and minimum amounts found in the individual samples, are summarized in table 35 in the order of their magnitude.

TABLE 35. AVERAGE INVERT SUGAR CONTENT OF THE DIFFERENT GROUPS AND MAXIMUM AND MINIMUM IN INDIVIDUAL SAMPLES

Group number	Character of organism	INVERT SUGAR Calculated to a moisture-free basis		
		Average	Maximum	Minimum
		%	%	%
14	Tin buckets	0.41	*	*
11	Last run, sweet	0.47	0.82	0.12
5	Failures	0.51	0.90	0.23
1	Control	0.60	1.58	0.19
15	Burned control	0.70	*	*
2	Incubator control	1.04	3.52	0.41
8	Fluorescent	1.09	2.46	0.38
3	Non-fluorescent	1.52	2.38	0.77
9	Composite	1.64	4.09	0.68
17	Sour sap, kept	1.88	2.83	1.23
16	Last run, sour	2.07	4.59	0.34
4	Pink cocci	2.45	7.90	0.65
6	Red yeasts	3.18	7.63	1.01
13	Green molds	3.56	4.39	2.61
10	Bacillus aceris	4.70	7.20	1.72
7	Gray yeasts	6.86	28.35	1.21
14	Wooden buckets	6.91	*	*
15	Pink yeasts	8.69	*	*
12	Fluorescent bacteria and spore-bearers	11.03	16.03	7.51

*Single sample.

It is readily seen that the average invert sugar figures show a fairly regular gradation from 0.41 to 11.03%, but that the maximum and minimum figures among the different groups exhibit wide variations. Thus the average for group 7, gray yeasts, is 6.86%, but it is obtained by averaging eight results, which vary all the way from 1.21 to 28.35%. These differences in the maximum and minimum figures found in the same group are doubtless due to the fact that certain strains produced a more complete infection and were better inverters of sucrose than were others. Certain of the organisms used, notably those of the fluorescent group, had but little effect on the sucrose. They

apparently feed mainly on the proteids and mineral salts and exert a detrimental influence on color and flavor. Generally speaking, the yeasts and molds, which often but not always thrive well in a slightly acid medium, together with the spore-bearing bacteria, had the most pronounced inverting action on sucrose, either through the production of invertase, or by the formation of acid, or both. In many cases they likewise seriously affected color and flavor.

Most of the remaining bacteria used in this work did not prove particularly active as invert sugar makers, but their harmful effect was in many instances manifested by the color and flavor of the sirup.

Discussion of the Total and Insoluble Ash and Malic Acid Values

A survey of the analytical data given in tables 16 to 34 shows that the ash and malic acid values of 34 of the samples examined were slightly below the standards used in determining the purity of maple products. These deficiencies have been noted in the discussion of the several groups. The question now arises whether these abnormalities, which in many cases are very slight, are due:

(1) To the exceptional conditions obtaining in these experimental trials of the manufacture of sirup from sap, e. g., the small amounts of sap evaporated, the small number of trees contributing to the individual samples, and the variation in density of the resulting sirup.

(2) To the treatment of the sirups after manufacture and previous to analysis.

(3) To the effect of the inoculating organisms on the physical characteristics and chemical composition of the sirups.

These considerations have been clearly explained in the preceding pages, but their connection with the analytical data under discussion has not been traced.

For the purpose of easy reference and to assign, if possible, a definite reason for these failures, all samples showing defi-

ciencies in ash or malic acid values are listed by groups in the following table.

TABLE 36. SIRUPS DEFICIENT IN TOTAL ASH, INSOLUBLE ASH, OR MALIC ACID VALUE

Group number	Character of organism	Sample number	Moisture	Sucrose	Invert sugar	CALCULATED TO MOISTURE-FREE BASIS			
						Total ash	Soluble ash	Insoluble ash	Malic acid value
			%	%	%	%	%	%	%
1	Control,	27	34.46	63.04	0.20	0.75	0.53	0.22	0.73
	"	58	36.30	61.80	0.28	0.73	0.43	0.30	0.83
	"	68	37.33	60.73	0.32	0.68	0.45	0.23	0.81
	"	89	29.96	67.11	0.24	0.61	0.32	0.29	1.01
	"	97	30.36	66.88	0.13	0.68	0.43	0.25	0.73
	"	113	29.99	67.89	0.43	0.75	0.50	0.25	0.73
2	Inc. cont'l,	96	27.81	68.82	0.32	0.71	0.46	0.25	0.65
	" "	104	29.46	68.42	0.29	0.69	0.47	0.22	0.60
	" "	112	31.34	67.01	0.76	0.71	0.50	0.21	0.44
	" "	120	38.97	58.06	0.35	0.71	0.45	0.26	0.72
4	P'k cocci,	107	27.90	68.15	0.50	0.71	0.50	0.21	0.58
	" "	31	38.21	59.70	0.19	1.14	0.55	0.59	0.55
5	Failures,	106	30.48	67.64	0.46	0.69	0.47	0.22	0.59
	"	108	29.64	67.10	0.63	0.65	0.45	0.20	0.49
6	R. yeasts,	114	30.69	66.75	0.70	0.71	0.49	0.22	0.56
	"	99	28.11	68.17	1.47	0.75	0.44	0.31	0.67
7	G. yeasts,	110	30.94	67.05	1.20	0.67	0.44	0.23	0.65
	"	115	31.04	65.61	1.05	0.72	0.51	0.21	0.62
8	Flu. bac.,	102	28.81	68.11	0.81	0.82	0.58	0.24	0.50
	"	103	28.75	67.97	0.96	0.73	0.45	0.28	0.60
	"	101	28.36	69.10	0.67	0.71	0.44	0.27	0.53
	"	100	27.76	69.59	0.67	0.79	0.54	0.25	0.55
	"	91	26.75	69.40	0.29	0.72	0.44	0.28	0.90
	"	93	28.06	68.65	0.31	0.83	0.56	0.27	0.54
	"	94	28.51	67.69	0.37	0.72	0.41	0.31	0.64
9	Comp'te,	116	29.89	67.04	0.48	0.86	0.65	0.21	0.65
	"	118	29.30	67.66	0.69	0.84	0.59	0.25	0.58
	"	119	28.75	68.59	0.58	0.64	0.42	0.22	0.59
11	Last run, sweet,	24	29.41	67.75	0.43	0.71	0.47	0.24	1.01
	"	122	32.55	64.61	0.45	0.73	0.45	0.28	0.76
	"	124	29.35	69.62	0.42	0.74	0.52	0.22	0.61
	"	128	31.81	64.23	0.21	0.59	0.29	0.30	0.82
	"	129	28.81	67.87	0.08	0.75	0.51	0.24	0.61
13	G. molds,	117	30.70	63.12	2.58	0.74	0.51	0.23	0.61
	Average,		30.62	66.50	0.57	0.74	0.48	0.26	0.66
	Maximum,		38.97	69.62	2.58	1.14	0.65	0.59	1.01
	Minimum,		26.75	58.06	0.08	0.59	0.29	0.20	0.44

This table, studied in conjunction with its predecessors, shows:

(1) That the color averaged 6, with extremes of 3 and 9, indicating that in all cases the goods were of high grade.

(2) That the flavor ranged from 1 to 5 with an average of 3, which corresponds to medium. Of the 34 samples listed, 4 scored 1 in flavor, 9 scored 2, 7 scored 3, 10 scored 4, and 4 scored 5 (buddy). In other words 20 samples or 3 out of 5 graded medium or better than medium.

(3) That 10 of the 34 samples were "controls," that 5 were "last run sap, sweet," that 1 was inoculated with green mold, 6 with yeasts, 8 with bacteria, and 3 with mixtures of yeasts and bacteria. Hence, 15, or 44% of the total number, were not artificially inoculated.

(4) That invert sugar was not formed in excessive amounts.

(5) That most of the samples contained far less water than the 35% which the standard 11 pounds to the gallon sirup carries. They were, consequently, considerably heavier than necessary. But 4 samples contained more than 35% of water, while thirty carried from 26.75 to 34.40%, and twenty less than 30% of water.

The standards of ash and malic acid used were predicated on an eleven-pound gallon, or 35% moisture, basis. It is quite evident that the over-concentration secured in these samples together with their thorough clarification before analysis, resulted in an increased precipitation and sedimentation of the "niter," greater than would have occurred had the sap sirup been concentrated only to the standard density. This tended to lower the ash and malic acid contents of the sample as analysed. This is strikingly brought out by a comparison of the analyses of samples containing over 34% water with those containing less than this amount as shown on the next page.

	No. of samples	Moisture	Calculated to a moisture-free basis: Total ash	Soluble ash	Insoluble ash	Malic acid value
Average,	128	34.63	0.93	0.44	0.49	0.90
Average above 34% water,	84	36.86	1.02	0.45	0.57	1.00
Average below 34% water,	42	30.05	0.80	0.48	0.32	0.71
Average below 30% water,	25	28.74	0.77	0.49	0.28	0.66

A gradual drop in the total and insoluble ash contents and in malic acid value occurs as the concentration increases. If averages show this condition, it is to be expected that the individual samples, which, in the instances under consideration, represent the sap from but a few trees and not a composite from the orchard, would show similar variations, some of which must of necessity be below the standard of comparison, which, of course, is based on commercial samples and not on such extreme conditions as obtained in this investigation.

In judging the purity of maple sirup, it is essential that the analyst should clearly understand the nature and significance of the data secured. The standard under consideration includes the total and insoluble ash contents and the malic acid value. They represent inorganic and organic constituents respectively. The slight departure of any one constituent from the standard should not in itself be interpreted too strictly, particularly if it be the total ash or malic acid value.

The number of samples below the standard, and the average, maximum and minimum deficiencies are stated in the following table.

TABLE 37. NUMBER OF SAMPLES AND PERCENT BELOW STANDARD IN TOTAL ASH, INSOLUBLE ASH AND MALIC ACID VALUE

CALCULATED TO MOISTURE FREE BASIS

Character of organism	Total ash				Insoluble ash				Malic acid value			
		Percent below				Percent below				Percent below		
	Below 0.77%	Average	Maximum	Minimum	Below 0.23%	Average	Maximum	Minimum	Below 0.60%	Average	Maximum	Minimum
Control,	6	.07	.16	.02	1	.01	...	...	0	...	...	...
Inc. con.,	4	.07	.08	.06	2	.01	.02	.01	1	.16	...	...
Non-fluo.,	0	...	...	...	0	...	...	...	0	...	...	...
P'k cocci,	1	.06	...	...	1	.02	...	...	1	.02	...	...
Failures,	2	.10	.12	.08	2	.02	.03	.01	3	.06	.11	.01
R. yeasts,	2	.04	.06	.02	1	.01	...	...	1	.04	...	...
G. yeasts,	2	.07	.10	.05	1	.02	...	...	0	...	...	...
Fluores't,	4	.05	.06	.04	0	...	...	...	4	.07	.10	.05
Comp'te,	1	.13	...	...	2	.01	.02	.01	2	.01	.02	.01
B. aceris,	0	...	...	...	0	...	...	...	0	...	...	...
Last run, sweet,	5	.07	.18	.02	1	.01	...	...	0	...	...	...
Fluor. bac. and spore-bearers,	0	...	...	...	0	...	...	...	0	...	...	..
G. molds,	1	.03	...	...	0	...	...	...	0	...	...	...
T. buck's,	0	...	...	..	0	...	...	...	0	...	...	...
W. buck's,	0	...	...	...	0	...	...	...	0	...	...	...
P. yeasts,	0	...	...	...	0	...	...	...	0	...	...	...
Burned control,	0	...	...	...	0	...	...	...	0	...	...	...
Last run, sour,	0	...	...	...	0	...	...	...	0	...	...	...
Sour sap, kept,	0	...	...	...	0	...	...	...	0	...	...	...
Total,	28				11				12			

Twenty-eight samples were deficient in total ash in amounts varying from 0.02 to 0.18%. The deficiencies mainly occur among samples in the control groups. Eleven samples were low in insoluble ash, but the shortages were very slight, varying from 0.01 to 0.03%, the latter figure occurring but once. There were 12 deficiencies in malic acid values, many of them being very small and ranging from 0.01 to 0.16%.

Inquiring still farther into these deficiencies, it will be found that in several cases but a single item of the three-fold standard is affected. Eliminating the groups exhibiting no departures from standard, the following table appears.

TABLE 38. NUMBER AND NATURE OF DEFICIENCIES

Group number	Character of organism	Low in total ash	Low in insoluble ash	Low in malic acid value	Low in total and insoluble ash	Low in total ash and malic acid value	Low in insoluble ash and malic acid value	Low in total ash, insoluble ash and malic acid value
1	Control	6	1	0	1	0	0	0
2	Incubator control	4	2	1	1	0	0	1
4	Pink cocci	1	1	1	0	0	0	1
5	Failures	2	2	3	0	0	0	2
6	Red yeasts	2	1	1	0	0	0	1
7	Gray yeasts	2	1	0	1	0	0	0
8	Fluorescent	4	0	4	0	1	0	0
9	Composite	1	2	2	0	0	0	1
11	Last run, sweet	5	1	0	1	0	0	0
13	Green mold	1	0	0	0	0	0	0
	Totals	28	11	12	4	1	0	6

Assuming that standard maintenance in two instances and a very close approach thereto in the third would suffice to pass a sample, a large share of the samples heretofore listed as below standard are eliminated and attention is fixed upon the 11 lots, listed in the last four columns, four of which are below in both total and insoluble ash, one low in both total ash and malic acid value, while six are low in all three items.

The following table has been prepared as an aid in locating the samples that show these deficiencies. The sample numbers correspond to those hitherto employed pages 352 to 390, reference to which will enable anyone interested to ascertain the entire history and chemical analysis of each lot.

TABLE 39. DEFICIENCIES GROUPED ACCORDING TO SAMPLE NUMBERS

Group number	Character of organism	Low in total ash	Low in insoluble ash	Low in malic acid value	Low in total and insoluble ash	Low in total ash and malic acid value	Low in insoluble ash and malic acid value	Low in total ash, insoluble ash and malic acid value
1	Control,	27, 28, 68 89, 97, 113	27 ..	.. ..	27 ..	.. ..	.. ..	.. ..
2	Incubator control,	96, 104, 112, 120	104, 112	112	104	..	..	112
4	Pink cocci	107	107	107	..	..	..	107
5	Failures,	106, 108	106, 108	31, 106, 108	..	..	..	108, 106
6	Red yeasts,	114, 99	114	114	..	..	..	114
7	Gray yeasts,	110, 115	115	..	115	..	..	..
8	Fluorescent bacteria,	103, 101 91, 94		101, 102 100, 93	..	101	..	..
9	Composite,	119	119, 116	119, 118	..	..	..	119
11	Last run, sweet,	24, 122, 124 128, 129	124 ..	.. ..	124 ..	.. ..	.. ..	.. ..
	Green molds,	117	..	..	..	..	..	..

Examining closely into the deficiencies of the 11 samples previously mentioned, it appears that Nos. 27, 104, 115 and 124 were more concentrated than the standard requires; yet, the deficiencies in total ash are but 0.02, 0.08, 0.05 and 0.03% respectively. In three cases the insoluble ash is but 0.01% and in the other but 0.02% below. They equal or exceed the standard in malic acid value.

No. 101 contains 28.36% water and is 0.06% low in total ash and 0.07% low in malic acid value, while, on the other hand, it is 0.04% over standard in insoluble ash. The remaining six samples Nos. 112, 107, 106, 108, 114, and 119, fall short in all

three items, the total ash ranging from 0.06% to 0.13%, the insoluble ash from 0.01% to 0.03% and malic acid value from 0.01% to 0.16% below standard requirements.

Explanatory of the deficiencies enumerated above, attention is now called to a procedure that was employed on 11 of the samples reported in table 36, in which are listed the samples below standard in any particular, and which, fortunately, included 5 of those now being considered. These 11 samples were selected at random before the previous compilations had been made and their choice, at that time, depended on the size of the sample available, together with the concentration as indicated by the moisture content.

The treatment to which they were submitted is known as the "boiling and filtering process" first suggested by the writer in 1905.[1] This, as was then pointed out, is an important procedure in the certain determination of the purity of a maple product. Suitable portions of water were added and the samples containing both sirup and precipitated "niter" were mixed, heated in a water bath at 65 to 75° C. for an hour, and allowed to settle for two days. A portion was then decanted and boiled in a beaker, until a thermometer inserted in the sirup indicated 104° C. The sirup was immediately filtered hot through double filter papers and the ash and malic acid value determined on the clear filtrate. The results obtained by this procedure are given in the following table together with those originally secured on the concentrated and clarified samples.

[1] Vt. Sta. Rpt. 18, p. 328 (1905).

TABLE 40. EFFECT OF DILUTING, BOILING AND FILTERING ON CONCENTRATED MAPLE SIRUP COMPARED WITH THE ORIGINAL ANALYSES OF THE SAME SAMPLES

Character of organism	Sample number	Treatment	CALCULATED TO A MOISTURE FREE BASIS						
			Moisture	Ash	Soluble ash	Insoluble ash	ALKALINITY: Soluble ash	ALKALINITY: Insoluble ash	Malic acid value
			%	%	%	%			%
Control,	105	B. & F. *	39.21	0.81	0.53	0.28	62	65	0.63
"	"	Original	30.53	0.79	0.54	0.25	58	48	0.61
"	113	B. & F.	37.25	0.96	0.55	0.41	64	51	0.75
"	"	Original	29.99	0.75	0.50	0.25	62	58	0.73
In. control,	104	B. & F.	35.40	0.77	0.43	0.34	62	59	0.74
"	"	Original	29.46	0.69	0.47	0.22	62	42	0.60
Pink cocci,	107	B. & F.	35.05	0.79	0.54	0.25	52	55	0.61
"	"	Original	27.90	0.71	0.50	0.21	57	34	0.58
Failures,	108	B. & F.	33.27	0.75	0.52	0.23	54	42	0.52
"	"	Original	29.64	0.65	0.45	0.20	58	37	0.49
Red yeasts,	114	B. & F.	35.75	0.92	0.65	0.27	58	53	0.62
"	"	Original	30.69	0.71	0.49	0.22	61	52	0.56
Gray yeasts,	110	B. & F.	35.62	0.81	0.56	0.25	56	59	0.61
"	"	Original	30.94	0.67	0.44	0.23	58	52	0.65
"	115	B. & F.	38.00	0.87	0.61	0.26	65	58	0.60
"	"	Original	31.04	0.72	0.51	0.21	61	53	0.62
Fluo. bac.,	91	B. & F.	35.95	0.78	0.51	0.27	59	59	0.61
"	"	Original	26.75	0.72	0.44	0.28	58	58	0.90
Gr. molds,	111	B. & F.	39.40	0.86	0.60	0.26	59	74	0.66
"	"	Original	28.29	0.77	0.53	0.24	61	57	0.67
"	117	B. & F.	35.75	0.87	0.53	0.34	62	65	0.64
"	"	Original	30.70	0.74	0.51	0.23	64	52	0.61

*Boiled and filtered.

The table shows conclusively that by simply diluting and boiling the entire sirup, including its niter, to its normal density, standards are met in every case. All the samples thus treated show an increased total ash content, sufficient to enable them to meet the standard requirement, save in the case of No. 108, the moisture content of which when boiled and filtered, was only 33.27%. The insoluble ash percentage has been increased in every case sufficient to fully meet the standard.

This treatment resulted in an increase in total ash for the 11 samples of 1.27%, averaging 0.115% for each sample, with extremes from 0.02 to 0.21%. The insoluble ash was increased 0.62%, averaging 0.056%, with extremes from —0.01 to +0.16%. The soluble ash was increased 0.55%, averaging 0.05%, with extremes from —0.04 to +0.16%. The standard is met as far as the malic acid value is concerned in all but one sample (No. 108) and even in this case the deficiency is slight.

This procedure seems to make it clear that the failure of several samples under discussion to meet standard requirements in certain particulars is not due to the influence of the inoculating organism employed, but rather to the over-concentration of the sample which, during the long period of sedimentation, caused a larger amount of niter to form and to settle out than would have formed and settled had the concentration been less and nearer that of the eleven-pound gallon.

SUMMARY

1. All inoculating organisms used (with a single exception, sirup number 12) had previously been obtained from maple sap and the so-called natural infection due to careless methods of gathering and handling may result in the introduction of any or all of the organisms employed in this investigation, with similar results as regards quality of product.

2. The conditions are more favorable to bacterial, yeast and mold contamination in the sap toward the close of the sugar season than earlier because of the higher temperatures, bare ground, rain, interrupted runs, less cleanly utensils, etc., which then obtain.

3. The relation of cleanliness in all operations and of promptness in collecting and boiling the sap to the maintenance of a high quality of product are strikingly shown by the results secured in this investigation.

4. The several forms of micro-organisms used in this study exerted apparently little or no effect on the ash content or the malic acid value of the sirups.

5. Slightly low ash contents and malic acid values were often obtained on individual samples of the better grades of sirup, which, however, represented the product from but a few trees. They occurred mainly when increased concentration beyond the eleven-pound gallon standard was followed by a prolonged sedimentation or thorough clarification.

6. The deficiencies noted in ash contents or malic acid values are not extreme and are due wholly to exceptional conditions of manufacture which would not obtain in commercial practice.

7. Standard maple syrup should weigh 11 pounds to the gallon, should carry between 34 and 35% of water, should give a Baumé reading of 35½ to 36 at 60° F., and should contain, calculated to a moisture-free basis, a total (maple syrup) ash of 0.77%, an insoluble ash of 0.23% and a malic acid value of

0.60%. These figures constitute the standard now in use for determining purity. They are none too low, should be considered collectively, and, when properly interpreted, should enable certain differentiation between pure and adulterated maple products.

PART III

TECHNICAL DESCRIPTION OF CERTAIN BACTERIA OCCURRING IN MAPLE SAP

By H. A. EDSON and C. W. CARPENTER

(A.) DESCRIPTION OF BACILLUS ACERIS (NEW SPECIES)

SUMMARY OF CHARACTERS

I. OCCURRENCE AND CHARACTER

The bacillus occurs as the causal organism in a certain type of stringy maple sap, in which it produces an acid reaction and milky appearance. It seriously affects the clearness and flavor of sirup and also causes an increase in the content of invert sugar.

II. MORPHOLOGY

1. *Form.*—A bacillus, with rounded ends, occurring singly or in chains.

2. *Size:*—.9 to 1 micron by 1.5 to 3 microns.

3. *Agar hanging block.*—Organisms occur as single rods or in long chains according to the amount of moisture present. Orientation of chains either parallel or irregular.

4. *Endospores.*—Not found.

5. *Motility.*—Very actively motile in liquid media and frequently also on solid media. Two to 7 peritrichiate flagella readily demonstrated by either Löwit's, Loeffler's or the Pitfield method.

6. *Capsule.*—Slight capsulation when grown in maple sap, milk or on certain carbohydrate agars.

7. *Involution forms.*—Vacuolation occasionally observed on potato.

8. *Staining reactions.*—Organisms stained readily with 1-10 watery fuchsin, gentian violet, carbol fuchsin, and Loeffler's alkaline methylene blue. Not stained by Gram's method.

III. CULTURAL FEATURES

1. *Agar.*

Stroke.—Moderate, filiform to echinulate.

Stab.—Filiform, becoming villous or plumose, surface growth abundant, often spreading

Plate.—Colonies round or slightly irregular, smooth becoming radiate or striate, convex, edge entire, undulate or lobate; internal structure granular to grumose.

2. *Gelatin.*

Stab.—Beaded, becoming villous and plumose. Liquefaction napiform, becoming infundibuliform, beginning in 18 days, complete in from 60 to 80 days.

Plate.—Colonies round or irregular, convex, edge entire, undulate or lobate, liquefaction absent.

3. *Nutrient broth.*—Strong transient clouding with pellicle formation. Sediment flaky or membranous, later viscid on agitation.

4. *Cooked potato.*—Moderate persistent growth, echinulate or spreading below, convex, glistening, contoured, slimy to butyrous. Odor of alcohol. Medium grayed.

5. *Milk.*—Acid, coagulation delayed, beginning in from 4 to 6 days. Coagulum not peptonized. Litmus milk first reddened, then reduced.

6. *Starch jelly.*—Growth copious, diastasic action feeble or absent. Reducing sugars not found, but alcohol formed.

7. *Silicate jelly containing Fermi's solution.*—Scanty growth.

8. *Cohn's solution.*—No growth.

9. *Uschinsky solution.*—Growth copious, the fluid becoming pronouncedly viscid.

10. *Sodium chlorid in bouillon.*—Growth in concentrations up to and including 9%, but not in 10%.

11. *Bouillon over chloroform.*—Growth unrestricted.

12. *Nitrogen.*—Obtained from peptone and asparagin.

IV. PHYSICAL AND BIOCHEMICAL FEATURES

1. *Gas production.*—Gas production in maple sap and in bouillon containing dextrose, sucrose, lactose, maltose, mannit, and potato extract, but not in bouillon containing glycerin. Gas composed of carbon dioxid and hydrogen.

2. *Growth in closed arm.*—Occurred in fermentation tubes of bouillon containing dextrose, sucrose, lactose, maltose, and mannit, and in tubes of potato extract, but not in tubes of bouillon containing glycerin.

3. *Acid production.*—Acid formed in maple sap, and in bouillon containing dextrose, sucrose, lactose, maltose, glycerin and mannit.

4. *Ammonia production.*—Moderate in bouillon.

5. *Nitrate reduction.*—Nitrates reduced to nitrites.

6. *Indol and phenol production.*—Feeble to moderate indol production in bouillon and in Dunham's solution. Phenol production negative.

7. *Toleration of acids.*—Medium growth in bouillon acidified with HCl to +25 Fuller's scale and in bouillon acidified to +20 with acetic acid.

8. *Toleration of sodium hydroxid.*—No growth in tubes of bouillon having a reaction more alkaline than −5 Fuller's scale. The organism was not killed in tubes having an initial reaction of −25, since, after twelve days, when the alkali was neutralized by atmospheric carbon dioxid, fair growth developed in such tubes. A more alkaline initial reaction invariably killed the organism.

9. *Optimum reaction.*—For growth in bouillon the optimum reaction was found to be +10 Fuller's scale.

10. *Vitality on culture media.*—Transfers from old cultures on various media, even when these had dried down, developed promptly.

11. *Temperature relations.*—Thermal death point in bouillon (10 minutes' exposure in water bath in thin walled tubes)

approximately 50 C. Optimum temperature 25° C. Maximum temperature 37° C. Minimum temperature not determined. Growth slow at 10° C.

12. *Desiccation.*—Growth occurred after drying for nine days in some cases, but not after drying for 20 days.

13. *Insolation.*—Exposure on ice to direct sunlight at noon in August for fifteen minutes killed 46.7% of the organisms.

14. *Acids produced.*—Not identified.

15. *Alkalies produced.*—Ammonia.

16. *Alcohols produced.*—Ethyl alcohol.

17. *Ferments produced*—The organism digests gelatin slowly; yet notwithstanding all attempts to demonstrate proteolytic ferments by the milk serum method gave negative results. Potato starch was evidently acted upon feebly, alcohol being produced, but diastatic ferments could not be demonstrated. Invertase formed in very small quantities.

18. *Effect of germicides.*—Formalin and phenol were tested in varying amounts in bouillon. Phenol retarded growth in dilutions of 1-1000 and killed the bacillus in dilutions of 1-500. Formalin retarded growth in dilutions of 1-2750 and inhibited growth in dilutions of 1-2200.

19. *Number.*—According to the numerical classification of the Descriptive Chart of the Society of American Bacteriologists the organism is *Bacillus* 221.1113022.

DETAILED DESCRIPTION

OCCURRENCE

At various times during the progress of the study of the bacteria of maple sap, stringy or ropy specimens of the material were encountered. These were of two types. The more common form was usually observed only after the close of the commercial season and was associated with the presence of filamentous fungi, yeasts, and various bacteria growing together. This sap always presented a more or less lumpy appearance when poured from the bucket. The other type of stringy sap was of a more uniform

consistency and was found only a few times during the progress of the studies.

Such a specimen of sap was first received in the laboratory during the sugar season of 1908. It possessed a decidedly stringy character, being so ropy that after turning a little of it from the flask in which it was received the material continued to siphon from the container, even when its mouth was turned up so as to be somewhat above the level of the sap within. The material was uniform in consistency, milky in color, and possessed a pronounced yeasty odor. Examination under the microscope revealed enormous numbers of actively motile bacteria apparently in almost pure culture. When plates were poured almost all the colonies developing were of one type. Cultures obtained from these were found capable of reproducing the characters of the original material when introduced into fresh maple sap. As explained under the head "Cultural Characters," page 493, the stringy property developed to a pronounced degree only in unsterilized sap, but since it always appeared after inoculation with this organism, and only when this organism was employed, and since the bacillus was always recovered from the inoculated material in practically pure cultures, there can be no doubt of its causal relation to the condition of the sap.

The organism has a very detrimental influence upon the quality of sirup. While the color is not always seriously impaired the sirup is made more or less cloudy, a very unnatural and unpleasant flavor is developed, and the amount of invert sugar is increased by the action of the organism.

FORM

The organism is a bacillus with rounded ends, occurring singly, in pairs, or often in filaments in young agar or nutrient broth cultures at 20-25° C. Short chains (5 to 7 segments) often occur in cultures 24 hours old and chains of 20 or more segments are not infrequent. The tendency to chain formation is less pronounced in the older cultures and persists longer upon solid media than in liquid cultures.

MORPHOLOGICAL CHARACTERS

DIMENSIONS

Stained specimens for measurement were prepared from young (24 hour) agar and broth cultures with watery solutions of the common anilin stains, with anilin water gentian violet, and with carbol fuchsin. Measurements using a Zeiss homogenous 1-12 objective and a number 3 micrometer ocular, or by photography, showed a diameter of from .9 micron to 1 micron, the majority being slightly less than 1 micron. The length varied from 1.5 microns to 3 microns. Living organisms upon agar hanging blocks measured by either method were found to have the same dimensions as the stained organisms with the possible exception of a few instances, in which there was an apparent length of 5 to 6 microns. Since careful focusing in some of these cases revealed indistinct lines of fission it is possible that the longer bodies are really filaments of 2 or more segments. (See Plate VIII, figs. 9 and 10).

CULTURES ON AGAR HANGING BLOCKS

Agar hanging block cultures were prepared from small blocks of nutrient agar cut from poured plates and placed on sterile cover glasses, the surface next to the cover glasses being first touched with a dilution from a young nutrient broth culture. The cover glasses were placed on hollow ground slides and sealed either with vaseline or with a bit of melted agar to prevent evaporation, and the preparation held at room temperature (20-24 C.) for observation under the microscope. In abundant moisture the grouping was similar to that described on page 479 under the heading "Form." In cultures too dry to afford easy locomotion, but not dry enough altogether to inhibit motion, long, variously oriented, vibrating or squirming chains were observed. (See Plate VIII, figure 8, in which the motion of the chains gave the negative a badly blurred appearance). Blocks in which motility was prevented by lack of moisture developed chains of from 2 to many segments, the elements usually showing parallel orientation but sometimes developing an irregular grouping.

PLATE IX.—Gelatin colonies of *Bacillus aceris*. Figures 1, 2, 3, and 4. Photograph of the same colony one, two, four, and seven days old. Figure 5. Colony seven days old, showing transition stage from entire to lobate edge. (See page 487.) × 75.

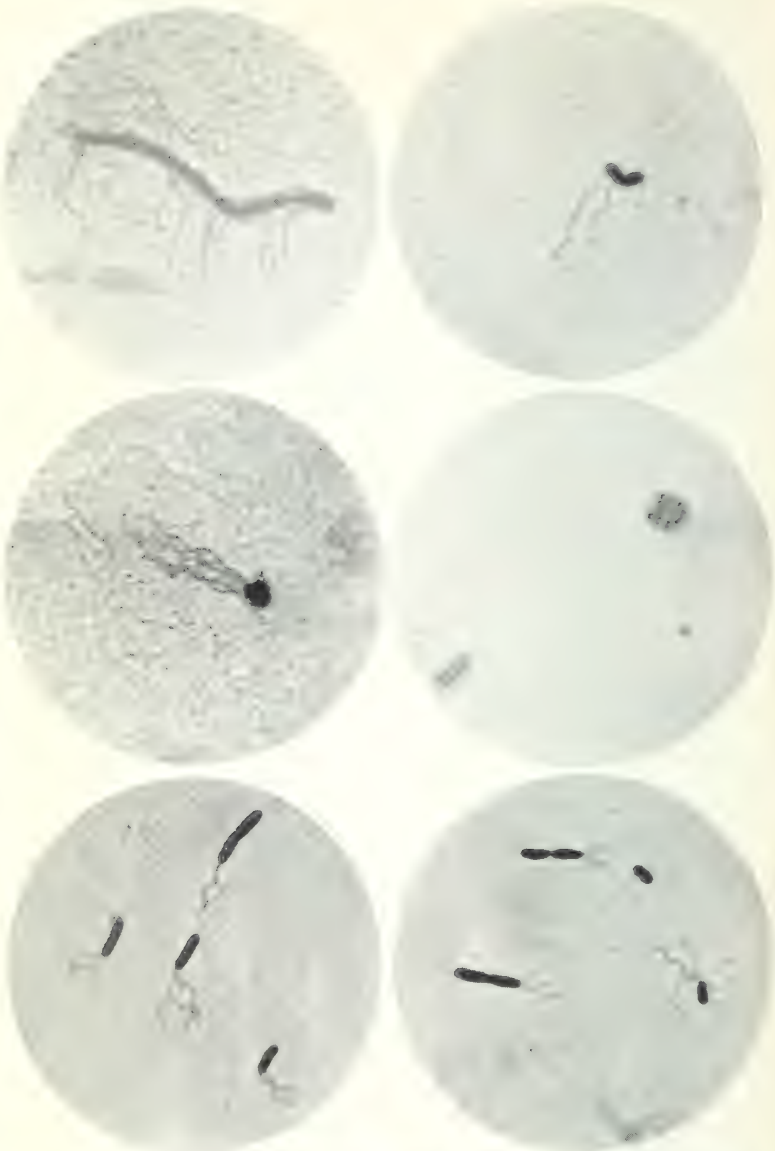

PLATE X. Flagella preparations from 24-hour agar slants (Loeffler's stain). Figs. 1-4 show capsulation.

Fig. 1. *B. aceris* Fig. 2. *B. parallelus*
Fig. 3. *B. parallelus* Fig. 4. *B. parallelus*
Fig. 5. *Ps. fluorescens*, strain CXLV Fig. 6. *Ps. fluorescens*, strain CXLV

(See pages 483, 554-556.) × 1500.

FISSION

The first indication of fission observed was a slight constriction at the center of organisms which were then 3 microns in length. The constrictions became gradually more pronounced and in the course of a few minutes two daughter cells each 1.5 microns in length were produced. These sometimes remained attached indefinitely while in other cases they separated almost at once. In the specimens observed the daughter cells showed no increase in length for a period varying from 20 minutes to 2 hours after division. There then occurred a period of rapid growth followed immediately by fission. The process of elongation and division were observed to take place in periods of from 20 to 40 minutes. Fission was completed in from 5 to 8 minutes after the first indication of its occurrence was observed. The active motility of this organism in abundant moisture made it impossible to carry out these studies under conditions of optimum humidity. The colonies under observation were so dry that growth continued only a few generations, and it is fair to assume that the rate of growth observed is far below the maximum. (Plate VIII, figures 1 to 5).

GROUPING

The formation of chains and filaments has been noted. Pseudo-zoogloca masses were observed in young cultures on broth, maple sap, and other liquid media.

MOTILITY AND FLAGELLA

Active motility occurred in young cultures in all liquid media employed. Colonies upon freshly poured plates of agar and gelatin exhibited internal motility under a Zeiss A objective and number 4 ocular. Motility became less marked in older cultures but was seldom entirely absent even in preparations made from cultures several weeks old, whether from liquid or from solid media. Flagella stains were obtained by Löwit's, Loeffler's, and the Pitfield methods, in preparations made from dilated condensation water of 24 hour old agar slant cultures. These

showed each rod to have from 2 to 7 peritrichiate flagella frequently developing a length of 15 microns. (Plate VIII, figure 6).

SPORES

No indications of spores were observed although search was made both by microscopic methods and by means of thermal death point determinations in old cultures upon agar and upon cooked potato.

CAPSULE

Microscopic examination of the mucilaginous deposit found in cultures upon maple sap as described under "Cultural Characters" revealed an envelope of almost transparent material and about 1 micron in thickness covering the organism. In unstained preparations, it was most readily observed by first placing the organisms in perfect focus with the diaphragm slightly open, and then reducing the light, when the colorless envelope became faintly though distinctly visible, presenting a well defined periphery. Organisms from the surface growth of carbohydrate agars showed a similar capsulation when mounted without the use of water. Organisms from carbohydrate agar condensation water or from milk showed a similar phenomenon, but the envelope was less clearly defined and scarcely more than .5 micron in thickness. Stained preparations from maple sap and milk cultures were disappointing. Sap preparations left to dry in the air formed a gelatinous mass about one-half the size of the original drop of culture placed on the cover slip, and the volume of this material could not be further reduced in drying with heat without charring the preparation. Richard Muir's capsule stain was tried repeatedly upon these preparations, but washing in water after the first mordant almost instantly dissolved the gelatinous mass and left the cover slips without a film of organisms. This difficulty was partially obviated by fixing in glacial acetic acid before the mordant and substituting a 2% salt solution for water in the subsequent washing. The stain, carbol fuchsin, was inactive

upon the material even when used with heat for 20 to 30 minutes, while the blue counter stain acted upon the organisms faintly. Part of the bacilli on the films thus treated showed an unstained envelope lighter than either the body of the organism or the field and from .5 to .75 micron in diameter. When Welch's capsule stain was employed the organisms from sap cultures appeared distinctly stained, surrounded by a transparent envelope .75 to 1 micron in thickness. Films from carbohydrate agar prepared by Welch's method showed a colorless envelope upon practically all organisms. Flagella preparations from 24 hour agar slants stained by Loeffler's method with anilin gentian violet often exhibited the capsule clearly stained. (Plate X, figure 1).

INVOLUTION FORMS

In a few cases oval refractive bodies suggestive of spores were seen in preparations from cooked potato cultures several weeks old. Transfers taken from such cultures were invariably killed by heating for 10 minutes at 55° C. and attempts to stain the bodies by the usual methods for spores gave negative results. These bodies were deemed to be vacuoles.

STAINING REACTIONS

Preparations made from 24 hour old cultures upon nutrient broth and nutrient agar were readily and deeply stained by cold watery solutions of the anilin dyes, by Ehrlich's anilin water gentian violet and by carbol fuchsin. Many of the rods stained by carbol fuchsin exhibited plasmolysis. It is an interesting fact that organisms from broth and agar cultures were deeply stained by exposure for 10 seconds to cold carbol fuchsin, while this stain used either cold or hot was totally ineffective with Richard Muir's method of capsule staining applied to organisms from sap cultures, as reported under the head "capsule." The organism was decolorized by the method of Gram.

CULTURAL CHARACTERS

METHODS

The culture media employed in this work were carefully prepared following closely the directions given in Smith's "Bacteria in Relation to Plant Diseases" and the publications of the American Public Health Association. Distilled water of a high degree of purity was used unless otherwise stated. The formula employed for preparing nutrient broth was 10 grams Witte's peptone, 5 grams of Liebig's extract of beef, and one liter of distilled water. Sodium chlorid was used only when so stated. All agar media contained 1.5% of agar flour. The reaction of media containing nutrient broth was +10 Fuller's scale unless otherwise noted. Titrations were made upon 5 cc. of medium diluted with distilled water to 50 cc. The reaction was determined in hot solution with N/20 sodium hydroxid against phenolphthalein. All transfers except those for determining spore formation were made from broth cultures 1 to 3 days old or from dilutions of the same in water or, for some special purposes, in liquid culture media. Transfers to fluid media were made with a 2 mm. platinum-iridium loop, and those to solid media with a straight needle. Exceptions are noted in special cases as they occur.

Agar stroke.—Cultures developed good growth within 24 hours, which was moderate in amount, varying from a filiform line to an echinulately bordered, rather broad band. The elevation varied from raised to broadly umbillicate, with a surface at first smooth but becoming faintly papillate and contoured in 4 or 5 days. Cultures 2 weeks or more old showed long villous or sometimes fleecy outgrowths into the substratum. The growth was translucent, accompanied by slight opalescence and a glistening luster. The culture was of slimy or butyrous consistency without definite color, developing no discoloration of the medium. The tubes gave off a mild yeasty odor.

Agar stab. Young stab cultures in agar showed a growth which at first was uniform along the line of puncture, later be-

coming better developed at the top, with a moderately spreading surface growth. Development along the line of puncture was at first filiform and then echinulate. Cultures 10 days old or more were often beset with scattered tufts of villous or plumose outgrowths usually more pronounced in the upper part of the medium.

Agar plates.—Colonies at 25° C. showed rapid growth. Those at the surface were at first round, sometimes becoming slightly irregular. In the early stages the surface was smooth, often becoming contoured and papillate or developing moderate striations and concentric markings. These characters were not infrequently combined in the same colony. The elevation was convex. The edge was at first entire or undulate, frequently becoming lobate. The internal structure of surface colonies showed all stages of variation from finely granular through coarsely granular to grumose and, less frequently, reticulated. The outer portion of many colonies was filamentous or curled. Young cultures often showed an even outer ring well differentiated from the body of the colony and consisting of motile chains through which were thickly scattered granules. The appearance suggested that which is often seen in very early stages of liquefaction on gelatin plates, but there was no sign of liquefaction in the agar. Young surface agar colonies 1 to 3 days old usually showed active motility within the colony. Buried colonies first appeared as brown lenticular bodies, often becoming irregular with the interior deeply reticulated.

Carbohydrate agars.—Cultures were made in shaken agar tubes containing 2% lactose, dextrose and sucrose, respectively. Just previous to sterilization 1% of a solution of azolitmin (one gram of azolitmin to 16 cc. of distilled water) was added to the tubes. The sterilized melted agar was cooled to 40° C., inoculated with a 2 mm. loop of a 24 hour old culture, thoroughly mixed by shaking and incubated at 25° C.

In lactose litmus agar good growth developed and acid production became evident during the first day. A heavy white film

of surface growth developed and during the third day gas production began. This continued to increase in amount for a few days and then ceased. The reaction remained acid and at no time was there evidence of reduction of the litmus.

In dextrose litmus agar the growth was more rapid than in agar containing lactose. The acid production was more pronounced and the gas formation observed was greater than was the case with either lactose or sucrose. Signs of bleaching at the surface appeared on the fifth day and progressed rapidly till the entire contents of the tube assumed a pale yellowish hue. The formation of a layer of liquid above the surface film of growth was noted in most of the tubes under observation.

In sucrose litmus agar tubes the organism developed very much as in dextrose tubes except that the growth and the various characters were slightly less pronounced, or at best developed a little later.

Gelatin.—All gelatin media contained 10% of Nelson's photographic gelatin No. 1 and was of such a consistency as to remain firm at 25° C. Gelatin cultures were incubated at 20° C.

Gelatin stroke.—Gelatin slants showed a beaded growth along the line of inoculation, frequently becoming filiform or echinulate, sometimes developing into a broad band with roundly dentate margins and spreading beneath into an arborescent growth. After a few days a fine filamentous outgrowth developed beneath the surface under the stroke and extended several mm. into the body of the gelatin. In some series this growth finally became arborescent while in others this character failed to develop to an appreciable extent. Liquefaction of gelatin began on the eighteenth day, and proceeded very slowly thereafter, becoming complete in about three months.

Gelatin stab.—Stab cultures in gelatin showed best growth at the top. The line of puncture was beaded, the beads soon united to form a granulose mass with a villous border developing into long capillary fibers extending nearly to the walls of the tube. These fibers eventually developed fine lateral branches,

thus producing a cloudy appearance. A characteristic surface colony was formed. The medium remained unchanged until the eighteenth to twentieth day when the first signs of liquefaction were observed. The action was very feeble but persistent. Tubes were completely liquefied in from 60 to 80 days. The cultures 30 days old exhibited infundibuliform or slightly napiform areas of liquefaction extending from the surface down about 15 mm. A heavy sediment deposited at the base of the liquefied portion which was clear above and covered by a firm, dry layer which had to be broken with a needle or by violent shaking in order to allow the liquefied portion to run out when the tube was inverted.

Gelatin colonies.—Growth upon gelatin plates developed rapidly at 20° C., producing colonies at first round and finally becoming deeply lobed under favorable moisture conditions. The elevation of growth was convex. During the early stages the colonies presented an entire edge becoming undulate the second day, and thereafter gradually becoming deeply lobate. The internal structure was at first finely granular becoming deeply reticulate or alveolar and developing a great variety of markings during the transition. Colonies one week old were strikingly characteristic, having a dark alveolar center surrounded by an intermediate lighter zone bearing finely reticulate markings, and verging into the outer zone consisting of deeply cut compact lobes composed of conglomerate aggregates, as if they had been formed by repeated expanding, bursting and reforming of an enveloping pseudomembrane. Liquefaction was not observed upon gelatin plates. (Plate IX).

Broth.—Cultures showed rapid growth and became semi-opaque from clouding in 24 hours at 25° C. The clouding increased for several days and was accompanied by the formation of a flaky or somewhat membranous pellicle. The sediment at first viscid became flaky in from 2 to 5 days. Cultures 6 to 8 weeks old were free from clouding with no pellicle but contained a sediment which was viscid on agitation. The broth at this age

took on a dark rich amber color and reacted distinctly alkaline to litmus.

Potato blocks.—Cultures upon cooked potato blocks showed a persistent growth which was moderate in amount, echinulate, often spreading at the base; convex with a glistening luster, contoured surface, and slimy to butyrous consistency. The tubes gave off a characteristic odor suggestive of alcohol, and gas production was noted. The medium was grayed.

Milk.—Tubes of fresh centrifuged milk inoculated with a 2 mm. loop of 24 to 48 hour old broth cultures held at 25° C. showed no visible change for 4 to 6 days. Thereafter coagulation occurred with gradual shrinking of the curd and extrusion of whey, accompanied by gas formation. The same changes were observed both at 20 and at 30° C., but the process was always slower at the lower temperature and more rapid at the higher. The coagulum was firm and leathery, becoming slightly yellowish by reflected light. Tubes held under observation for 2 months showed yellowish translucent spots which suggested slight peptonization, but they were believed to result merely from shrinking of the curd. (See production of proteolytic enzyms, pages 509).

Acid production in milk.—This was determined in the following manner: Freshly drawn milk was centrifuged, then further freed from fat by filtering through several thicknesses of filter paper, carefully pipetted into tubes, 10 cc. in a tube, sterilized by discontinuous steaming on 3 consecutive days, and inoculated with a 2 mm. loop from young broth cultures. Titrations were made at the end of 1, 2, 4, 10, and 20 days, respectively. For this purpose the content of the tube was added to 40 cc. of distilled water, the mixture boiled for 1 minute and at once titrated against phenolphthalein with N/20 sodium hydroxid. The acid production was calculated by subtracting from the reaction of the inoculated tube the average reaction of all check tubes in the same series and averaging the differences thus obtained.

TABLE 41. ACID PRODUCTION IN MILK

Age of culture	Series 1, 20° C. Reaction in Fuller's scale inoculated tubes	control	Acid production in cc. N/1 per liter	Series 2, 20° C. Reaction in Fuller's scale inoculated tubes	control	Acid production in cc. N/1 per liter
1 day,	19.7	12.0		15.0	14.6	3.0
1 day,	19.2		7.3	18.2		
2 days,	28.3	9.9		25.0	13.3	10.7
2 days,	25.6	12.5	14.6	23.6		
4 days,	39.4	12.4		26.7	13.2	13.7
4 days,	33.6	10.1	24.2	28.0		
10 days,	46.6	13.6		36.7	14.0	25.8
10 days,	39.6	15.0	30.9	42.1		
20 days,	49.1	11.4		lost		
20 days,	41.4	12.9	33.0	lost		
Average of controls,		12.20			13.6	
	Series 3, 25° C.			Series 4, 25° C.		
1 day,	28.8	17.8		27.5		
1 day,	27.8	17.8	9.6	27.8	18.4	8.5
2 days,	32.3	20.0		34.0		
2 days,	32.6	20.5	13.3	30.5	18.8	13.1
4 days,	32.0	19.5		37.8		
4 days,	32.0	19.8	12.8	38.3	20.0	18.9
10 days,	37.0	18.8		48.3		
10 days,	37.0	19.0	17.8	46.3	19.6	28.1
20 days,	48.3	19.0		54.5		
20 days,	46.8	19.4	28.4	52.0	19.2	34.1
Average of controls,		19.2			19.2	
	Series 5, 25° C.			Series 6, 25° C.		
1 day,	18.7	10.2		20.0	13.7	8.0
1 day,	18.4	8.8	8.4	22.7		
2 days,	27.1	9.1		28.0	12.5	13.7
2 days,	26.9	....	16.8	26.0		
4 days,	27.1	9.1		35.5	12.8	22.9
4 days,	27.1	8.5	16.9	37.0		
10 days,	39.9	12.1		40.0	14.2	28.4
10 days,	38.3	....	28.9	45.5		
20 days,	45.4	12.1		48.0		
20 days,	48.3	11.7	36.7	48.6	13.3	35.0
Average of controls.		10.2			13.3	

ACID PRODUCTION—*Continued*

Age of culture	Series 7, 25° C. Reaction in Fuller's scale inoculated tubes	control	Acid production in cc. N/1 per liter	Series 8, 30° C. Reaction in Fuller's scale inoculated tubes	control	Acid production in cc. N/1 per liter
1 day,	23.9	12.6	10.6	26.2	10.0	13.8
1 day,	21.2	10.6		25.3	....	
2 days,	29.1	10.9	14.0	28.6	12.2	18.8
2 days,	22.9	12.3		32.9	12.8	
4 days,	42.9	11.9	24.7	37.8	15.6	27.5
4 days,	30.4	12.7		41.1	10.6	
10 days,	46.6	12.2	33.9	48.5	13.8	31.9
10 days,	45.0	....		39.2	13.6	
20 days,	50.5	11.4	38.6	54.5	13.2	43.3
20 days,	....	12.9		56.0	15.9	
Average of controls,		11.93			11.94	

Age of culture	Series 9, 30° C. Reaction in Fuller's scale inoculated tubes	Controls	Acid production in cc. N/1 per liter
1 day,	24.7	13.5	11.8
1 day,	26.2		
2 days,	33.5	13.0	21.1
2 days,	36.0		
4 days,	42.4	14.0	27.1
4 days,	39.0		
10 days,	44.0	14.2	30.7
10 days,	44.7		
20 days,	57.8	14.3	43.9
20 days,	58.6		
Average of controls,		13.8	

TABLE 12. AVERAGE ACID PRODUCTION IN MILK IN CC. N/1 PER LITER

	20° C.	25° C.	30° C.
1 day,	5.15	9.02	12.80
2 days,	12.65	14.18	19.95
4 days,	18.95	19.24	27.30
10 days,	28.35	27.42	31.30
20 days,	33.00	34.56	43.60

Litmus milk was prepared by adding 0.10% of azolitmin to fresh centrifuged milk and filtering through several thicknesses of filter paper. Acid production became apparent within 24 hours at 20, 25 and 30° C. followed by gas production, coagulation, and extrusion of the whey, as reported under milk. Gradual but complete reduction of the litmus was observed beginning at the top of the tube about the sixth day and becoming complete about the sixteenth day. No further change was observed in tubes held under observation for 3 months.

Starch jelly was prepared according to the directions given by Smith. The nutrient material employed was the same author's modification of Uschinsky solution. Good growth occurred upon this within 24 hours. A broad band of raised growth, higher at the border than at the center, with a papillate surface, an echinulate edge and a faint opalescence, resulted. Slight local liquefaction of the medium was noted on the sixth day accompanied by a change from opalescence to opaqueness with gas production in the medium. On the tenth day and at intervals thereafter for six weeks, tubes were examined by washing with distilled water. Portions of the starch jelly had been rendered soluble so that pockets were left in the surface. The wash-water and dissolved material was passed through filter paper and tested for sugar with Fehling's solution, with negative results. A portion of the filtrate was distilled and the distillate tested with sodium hydroxid and iodine as described under alcohol production. Iodoform was produced, indicating the presence of alcohol, aldehyde, or ketone. From the fact that Fehling's solution was not reduced the probability is strongly in favor of the supposition that the body was an alcohol.

Cohn's solution proved an unfavorable medium for the cultivation of this organism. Cultures held under observation for 21 days failed to show growth.

Uschinsky solution.—Cultures developed a copious growth appearing within 24 hours, accompanied by a pellicle formation which after about 3 days sank and deposited as a membranous

sediment. The medium was slightly viscid at the end of 2 weeks.

Fermi's solution inoculated with organisms developed a slight growth within 24 hours, followed by the formation of a thin pellicle which sank and formed a thin membranous sediment. The growth was transient, the tubes becoming almost clear in 3 days.

Silicate jelly containing Fermi's solution showed a light growth, becoming apparent the second or third day as a thin, pearly white, surface development. The medium and growth thereafter remained unchanged.

Sodium chlorid in bouillon.—Transfers were made to bouillon (+10 Fuller's scale) containing 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12%, respectively, of chemically pure sodium chlorid. Growth promptly appeared in the four weaker solutions, but was very slightly restricted in 5% sodium chlorid. It invariably appeared upon the 6% and 7% solutions within 4 days and upon the 8% and 9% ones in from 3 to 7 days. No growth occurred in 10%, 11%, or 12% sodium chlorid bouillon within 21 days. Transfers from these tubes into normal bouillon gave no growth. In no case was development entirely inhibited by less than 10% of sodium chlorid, but cultures in solutions containing from 5% to 9% were not characteristic. Microscopic examination showed organisms to be grouped together in chains in the 5% solution. No signs of motility were observed in this or stronger concentrations. In the broths containing in excess of 5% the growth was restricted to clustered colonies gathered on the sides of the tubes. In solutions up to 4% growth was apparently normal, the grouping and motility being characteristic.

Growth in bouillon over chloroform was unrestricted and characteristic.

Maple sap sterilized by heating at 99 to 100° C. on three consecutive days and inoculated with a 2 mm. loop of the organism, developed a deep milky appearance within 24 hours. Growth was accompanied by the formation of mucilaginous gum on the walls of the flask in contact with the culture fluid. This layer

increased in amount for one or two days, but began to diminish on the fourth day and was entirely gone on the sixth day. Meantime the milky appearance of the sap remained unchanged. A slimy consistency developed and the material sometimes became stringy, but the pronounced ropy character described under cultures in unsterilized sap did not appear.

Unsterilized sap was employed in a large number of the inoculation experiments. Care was taken to procure this fresh from the trees and as slightly contaminated as possible by bits of dust, bark, and other extraneous matter, in order that the natural inoculations should be minimized. In the first series a young culture in beef broth was employed for the inoculations, about 5 cc. of culture being added to a liter of sap; in other series varying small amounts of broth culture were employed, and in still others pure cultures upon sterilized sap were used to produce the inoculations. The cultural characters were invariably the same, except that the heavier inoculations produced the results more quickly. The sap took on a deep milky appearance but developed no pellicle. A slime was deposited upon the walls of the containing vessel but the gummy consistency of the deposit described under sterilized sap was not observed. Moreover the liquid developed a ropy character which was very pronounced after 24 to 48 hours and persisted for the two weeks that the cultures were held under observation. The character of the culture produced by inoculation with the organism after it had been cultivated in the laboratory for one year was exactly similar to that observed in the original material from which the organism was isolated. The maximum reaction observed in unsterilized sap in which this organism was cultivated for three days was +.17 Fuller's scale. Before inoculation the reaction was +.3. Associated organisms, when introduced in maple sap in such quantities as to cause an overgrowth, produce a reaction of +.6 to +1.

Artificial maple sap was prepared from maple sirup as follows: 25 cc. of sirup, 475 cc. of water and 25 cc. of nutrient bouillon were mixed, heated to boiling, filtered through filter

paper and sterilized in flowing steam on each of three consecutive days. When inoculated this medium clouded promptly, becoming milky. Ten day old cultures were slightly opalescent and very milky, but neither pellicle nor sediment developed. The reaction as shown by titration against phenolphthalein was +.81 Fuller's scale. Fermentation tubes filled with this medium showed equally good growth in the open and closed arms, characterized by a deep milky appearance and moderate gas production. The material became viscid and sometimes a little stringy. From 5 to 15% of the closed arm was filled with gas consisting of carbon dioxid and hydrogen.

PHYSICAL AND BIOCHEMICAL FEATURES

Gas production in milk.—Fermentation tubes containing milk inoculated with a 2 mm. loop of a young culture of the organism and plated at 20° C. showed a small bubble of gas in the closed arm at the end of two days; otherwise the milk was unchanged in appearance. Held at 25° C., the tubes of the same age showed gas in 8% of the closed arm, while those held at 30° contained from 15 to 20%. On the third day at 20°, 5% had developed, at 25°, 15 to 20%, and at 30°, 40 to 45%. The appearance of the milk still remained unchanged. Cultures 10 days old showed varying amounts of gas up to 100% of the closed arm. The milk was coagulated and the firm coagulum usually remained in the closed arm attached to one side of the tube. About 70% of the gas produced was absorbed by sodium hydroxid and the remainder gave a slight explosion upon ignition in air.

Carbohydrate broth.—Fermentation tube cultures of nutrient bouillon containing dextrose, sucrose, lactose, maltose, glycerin, and mannit were observed for gas production and acid production. Titrations were made at the end of the first, second, and fourth days, using 5 cc. of the culture in 45 cc. of boiling water against phenolphthalein. Dextrose tubes showed rapid development of the organism. At 20° the tubes became cloudy through-

out within 15 hours after inoculation, while at 25 and 30 gas production was well under way. The growth in lactose and sucrose was similar to that in dextrose but less pronounced. Maltose was especially favorable for the development of the organism and abundant gas production was obtained upon this sugar. Glycerin broth appeared a good medium for growth in the open arm, but only restricted growth occurred in the closed arm, and no gas was produced. Mannit gave good growth in both the open and closed arms accompanied by gas production. The tables showing acid production follow.

TABLE 13. ACID PRODUCTION IN CARBOHYDRATE BROTH

Age of culture	Reaction in Fuller's scale: Inoculated tubes	Acid production in cc. N/1 per liter	Inoculated tubes	Acid production in cc. N/1 per liter	Inoculated tubes	Acid production in cc. N/1 per liter
	Series 1-3. Dextrose					
	At 20° C.[1]		At 25° C.		At 30° C.	
1 day,	14.0	4.0	17.0	7.0	20.0	10.0
1 day,	15.0	5.0	18.0	8.0	20.0	10.0
2 days,	21.2	11.2	23.0	13.0	22.5	12.5
2 days,	21.2	11.2	24.5	14.5	24.0	14.0
4 days,	19.5	9.5	21.5	11.5	15.3	5.3
4 days,	19.5	9.5	21.2	11.2	16.0	6.0
	Series 4-6. Sucrose					
	At 20° C.[2]		At 25° C.		At 30° C.	
1 day,	14.5	5.0	16.5	7.0	19.5	10.0
1 day,	14.5	5.0	17.0	7.5	19.5	10.0
2 days,	16.2	6.7	20.0	10.5	19.5	10.0
2 days,	17.2	7.7	19.0	9.5	10.3	10.8
4 days,	17.5	8.0	16.5	7.0	16.8	7.3
4 days,	18.5	9.0	16.5	7.0	17.7	8.2
	Series 7-9. Lactose					
	At 20° C.[3]		At 25° C.		At 30° C.	
1 day,	12.0	2.5	16.5	7.0	20.0	10.5
1 day	11.0	1.5	17.5	8.0	20.0	10.5
2 days,	15.5	6.0	16.8	7.3	19.0	9.5
2 days,	15.5	6.0	16.7	7.2	18.0	8.5
4 days,	16.0	6.5	18.0	8.5	18.0	8.5
4 days,	17.0	7.5	19.0	9.5	18.5	9.0

[1] Average of controls, 10.0.
[2] Average of controls, 9.5.
[3] Average of controls, 9.5.

TABLE 13—*Continued*

Age of culture	Reaction in Fuller's scale: Inoculated tubes	Acid production in cc. N/1 per liter	Inoculated tubes	Acid production in cc. N/1 per liter	Inoculated tubes	Acid production in cc. N/1 per liter
	Series 11-12. Maltose					
	At 20° C.[4]		At 25° C.		At 30° C.	
1 day,	14.2	2.8	18.3	6.9	17.5	6.1
1 day,	15.7	4.3	18.7	7.3	16.5	5.1
2 days,	16.2	4.8	16.5	5.1	18.0	6.6
2 days,	16.5	5.1	16.5	5.1	17.6	6.2
4 days,	19.6	8.2	18.9	7.5	18.2	6.8
4 days,	17.8	6.4	17.6	6.2	19.0	7.6
	Series 13-15. Glycerin					
	At 20° C.[5]		At 25° C.		At 30° C.	
1 day,	9.0	—0.6	8.2	—1.4	9.7	0.1
1 day,	9.3	—0.3	8.5	—1.1	10.5	0.9
2 days,	11.2	1.6	10.3	0.7	10.9	1.3
2 days,	11.2	1.6	10.5	0.9	10.9	1.3
4 days,	11.0	1.4	11.1	1.5	13.7	4.1
4 days,	11.7	2.1	11.4	1.8	13.7	4.1
	Series 16-18. Mannit					
	At 20° C.[6]		At 25° C.		At 30° C.	
1 day,	12.0	4.0	14.0	6.0	15.0	7.0
1 day,	14.0	6.0	12.8	4.8	15.1	7.1
2 days,	14.7	6.7	16.1	8.1	17.8	9.8
2 days,	15.3	7.3	15.7	7.7	16.0	8.0
4 days,	16.4	6.4	16.8	8.8	19.6	11.6
4 days,	17.0	9.0	17.9	9.9	20.2	12.2

[4] Average of contents, 11.4.
[5] Average of controls, 9.6.
[6] Average of controls, 8.0.

The rate of gas production in the closed arms of the Smith tubes was followed by measuring with the Frost card. The amount of carbon dioxid formed was determined approximately by absorbing it within the tube with sodium hydroxid, the volume being noted before and after absorption. The residue was subjected to the flame test for hydrogen. A positive reaction invariably resulted, although the explosion was frequently so faint as to arouse suspicion that the material tested might be a mixture of gases rather than pure hydrogen. The tabulated results follow. The figures express the percents of the closed arm of Smith tubes which were filled with gas.

TABLE 44. GAS PRODUCTION IN CARBOHYDRATE BROTHS

Age	Temperature	Dextrose	Lactose	Sucrose
1 day,	20° C.	trace	0	0
	25° C.	5%	trace	5%
	30° C.	15%	8%	14%
2 days,	20° C.	15%	3%	8%
	25° C.	25%	20%	35%
	30° C.	40%	25%	35%
4 days,	20° C.	35%	25%	25%
	25° C.	45%	40%	30%
	30° C.	45%	48%	50%

TABLE 45. PROPORTION OF CARBON DIOXID TO TOTAL GAS

	20° C.		25° C.		30° C.	
	Percent of gas produced	Percent CO_2 to total	Percent of gas produced	Percent CO_2 to total	Percent of gas produced	Percent CO_2 to total
	Series 1. Four days old					
Dextrose,	35	43	45	44	45	55
Lactose,	25	20	40	30	48	35
Sucrose,	25	40	30	45	50	50
	Series 2. Fourteen days old					
Dextrose,	29	62	56	50	48	45
Ditto,	39	59	63	44	45	57
Ditto,	40	60	51	58	..	..
Lactose,	37	75	48	58	36	72
Ditto,	40	72	35	63	41	61
Ditto,	40	70	55	60	31	77
Sucrose,	26	50	49	53	48	..
Ditto,	25	48	27	70	34	62
Ditto,	26	54	57	49	41	53

Fermentation tubes of nutrient broth containing 2% of mannit showed gas production in 24 hours at 25° C. but not at 20°. At the end of two days 10% of gas had developed at 20° while at 30° 25% had formed. Cultures six days old showed a development of about 40% of gas, approximately one-half of which was absorbed by sodium hydroxid. Evidences of fermentation of maltose at 20° were pronounced in 15 hours, progressing rapidly till 40 to 50% of gas was formed, which was similar in composition to that produced upon other sugars. No gas was produced upon glycerin broth.

Fermentation of potato starch.—Potato fermentation tubes were prepared by placing cylinders of potato in the closed arm and filling with distilled water. The tubes were sterilized by steaming on each of three consecutive days, and inoculated with a 2 mm. loop from young broth cultures. Gas production occurred within 15 hours after inoculation in tubes held at 30°. At the end of three days tubes kept at 20° showed 25 to 35 percent of the closed arm filled with gas. The gas was tested with sodium hydroxid and the flame test and found to contain carbon dioxid and hydrogen.

Potato extract was prepared by boiling potatoes one-half hour in distilled water and decanting the liquid upon a filter. Fermentation tubes of the medium developed good growth. Gas consisting of carbon dioxid and hydrogen was produced by the second day. Tests for reducing sugars and for aldehydes made on the third day negative results. These ingredients were sought by the Fehling solution and ammoniacal silver nitrate methods, respectively. Potato extract to which 4% of sucrose was added, developed a remarkable growth. The fluid became milky and slightly stringy and developed a yeasty odor very much like that produced in sap.

Gas production in modified Uschinsky solution containing carbohydrates.—To modified Uschinsky solution 2% of the following carbohydrates were added, maltose, dextrose, sucrose, lactose and mannit. The media were placed in fermentation

tubes, sterilized by flowing steam on each of three consecutive days, and inoculated in the usual way. At the end of 8 days gas production in the closed arm was as follows: Maltose 22%, dextrose 15%, sucrose 12%, lactose 5%, and mannit 14%. The composition of the gas was similar to that previously described. Gas production in maple sap and in starch jelly containing modified Uschinsky solution has been mentioned under cultural characters.

Ammonia production was determined in 100 cc. portions of nutrient broth in 500 cc. flasks inoculated with 1 cc. portions of young cultures and incubated at room temperatures, 20 to 23° C. Determinations were made at the end of 5, 10 and 12 days. For this purpose an excess of heavy magnesium oxid and 100 cc. of distilled water were added. The flask was connected with a Liebig condenser and distilled, the distillate being received in a flask containing a measured quantity of N/20 hydrochloric acid. The excess of acid was titrated against cochineal as an indicator and the ammonia production calculated. An equal volume of the same broth which had been kept under identical conditions was analyzed for ammonia at the same time. The results are recorded in the following table:

TABLE 46. AMMONIA PRODUCED IN NUTRIENT BROTH

Age	cc. N/20 HCl neutralized				cc. N/20 NH_3 produced per 100 cc. broth
	Inoculated		Control		
5 days,	11.80	—	2.00	=	9.80
10 days,	36.20	—	4.20	=	32.00
12 days,	33.15	—	4.75	=	28.40
12 days,	28.25	—	4.25	=	24.00

Nitrate reduction.—Nitrate broth was prepared according to the following formula: One liter distilled water, 3 grams Liebig's extract of beef, 10 grams chemically pure potassium nitrate. The reaction was adjusted to zero with sodium hydroxid. The organism made vigorous growth upon this medium as exhibited by the prompt clouding and production of sediment. There was a marked tendency towards clearing in the upper

portion of the medium. Cultures were grown at 20, 25, and 30° C. and tested for nitrites at the end of 3, 5, 10 and 25 days with prompt and positive reaction. The test was made with the following solutions:

(1) One gram of potato starch boiled in 100 cc. of distilled water. (2) One-tenth gram of potassium iodid in 25 cc. of distilled water. (3) Two parts of chemically pure sulphuric acid and one part water. To the tubes to be tested 1 cc. of number 1, and 1 cc. of number 2, and, then, three drops of number 3 were added, the tubes being agitated after each addition.

Indol production.—The tests for indol production were made in Dunham's peptone solution and in sugar free bouillon. Both media were well suited to the growth of the organism. The test was made by adding 10 drops of chemically pure sulphuric acid (two parts sulphuric acid to one part water). After allowing the tubes to stand for 20 minutes to determine the absence of reducing bodies, 1 cc. of 0.02% sodium nitrite solution was added. Cultures 10 days old on Dunham's peptone solution gave a pink color in from 1 to 5 minutes after the addition of the nitrite. Cultures grown at 20° responded somewhat more slowly and with a fainter reaction than those which were held at higher temperatures. As a check upon the work, tubes of the same medium were inoculated with a strain of *B. coli* which produced indol vigorously, and held at 30° C. Taking the color obtained from these tubes as a standard, represented numerically by 10, cultures of the organism held at 30° produced a color of 5 within one minute. Cultures held at 20° produced a color of 1 after 15 or 20 minutes. A large number of cultures were tested in both Dunham's solution and in sugar free bouillon and positive, though sometimes faint, reaction was obtained in every case.

Production of phenol.—Tests for the production of phenol were made upon 50 cc. portions of 10 day old broth cultures of the organism. The material was transferred to a distilling flask, 5 cc. of concentrated hydrochloric acid added, and about 20 cc. of distillate collected. This was divided into three portions.

One was treated with a few drops of Millon's reagent,[1] another with two or three drops of dilute solution of ferric chlorid, and the third with a few drops of strong bromine water.[2] The results were negative.

The reaction for phenol is sometimes obscured by the presence of traces of indol. To eliminate this source of error 200 cc. cultures were distilled with 50 cc. of concentrated hydrochloric acid. The 70 cc. of distillate obtained was rendered alkaline with potassium hydroxid and again distilled. Indol should come over in the distillate while phenol remains in the residue. The residue was cooled, saturated with carbon dioxid and distilled. This final distillate was tested as before for phenol with negative results.

Hydrogen sulphid production.—This was determined in bouillon tubes in the upper portion of which were suspended strips of filter paper moistened with lead acetate solution. The moisture was renewed with distilled water as was deemed necessary. No signs of hydrogen sulphid appeared until the fourth day, when traces of the black sulphid of lead appeared upon the moist paper. The reaction became more pronounced as the age of the culture increased.

Toleration of acids.—Ordinary beef bouillon having a reaction of 0 Fuller's scale was used in this work. Hydrochloric and acetic acids were tested. Sufficient normal acid was added to 100 cc. portions of bouillon to secure a reaction of +5, +10 and so on for every 5 degrees Fuller's scale up to +50. Good growth resulted within 24 hours in tubes containing hydrochloric acid up to and including +20. Growth was observed in tubes having a reaction of +25 at the end of 2 days, and in 2 of the

[1] Prepared by heating one part of mercury with two parts of nitric acid, specific gravity 1.4, until the mercury was completely dissolved, and then diluting the solution obtained with twice its volume of distilled water. Salkowski & Orndorff. Laboratory Manual of Physiological and Pathological Chemistry, p. 251 (1904).

[2] Chester, F. D. Manual of Determinative Bacteriology, p. 33 (1901). Salkowski & Orndorff, pp. 106 and 161.

tubes having a reaction of +30 at the end of 4 days. No further change occurred thereafter during the 19 days the tubes were held under observation. Similar results were obtained upon the second series, growth being obtained upon 3 out of 5 tubes having a reaction of +30. In the third series 4 out of 5 tubes having a reaction of +30 showed growth. The organism was less resistant to acetic than to hydrochloric acid. Feeble growth occurred in 2 out of 5 of the tubes having a reaction of +20 Fuller's scale, and in the weaker solutions of acetic acid the growth was delayed and restricted.

Tolcration of sodium hydroxid.—For this work 100 cc. portions of nutrient bouillon having a reaction of 0 Fuller's scale were measured out and to each was added the theoretical amount of N/1 sodium hydroxid to produce reactions of —5, —10, etc., every 5 degrees of Fuller's scale up to —45. The tubes were inoculated as soon as possible after sterilization under pressure, placed at room temperature, 20 to 24° C., and held under observation. Growth was observed at the end of 24 hours in tubes calculated to have a reaction of —5, and in one tube calculated to have a reaction of —10. At the end of 4 days growth was visible in all tubes calculated to have a reaction of —15, and in 2 out of 4 tubes calculated to have a reaction of —20. At the end of 7 days growth was observed in all tubes calculated for —20. At the end of 12 days growth was observed in all tubes calculated to have a reaction of —25. Thereafter no change occurred during the 19 days the tubes were held under observation. Transfers made from tubes which developed no growth after 19 days showed them to be sterile. Sterile control tubes of this broth calculated to have a reaction of —25, —30 and —35 were titrated at the end of 20 days and found to have a reaction of from —19 to —14. This change in reaction is of course to be attributed to the formation of sodium carbonate from the carbon dioxid of the atmosphere. The results however show that the organism is killed when introduced to beef broth medium containing sufficient sodium hydroxid to give a reaction of some-

thing less than —30 Fuller's scale, and that growth is at least slightly restricted in broth containing sufficient sodium hydroxid to give a reaction of —10. The second series of tubes was prepared in the same manner as those already described, inoculated, and placed in sealed glass jars containing a few cc. of 2% sodium hydroxid. At the end of 10 days the jars were opened and the tubes examined for growth. Those calculated to have a reaction of —5 all showed healthy development, but no growth was observed in tubes having a more alkaline reaction. Titration of a control tube of the —5 broth showed that the reaction had not fallen below —4 Fuller's scale. Tubes calculated to have a reaction of —10 tested —6.1 and —6.8 and those calculated to be —15 tested —10 and —11.

The third series was prepared in the same manner as the second, but was incubated in Novy jars filled with air from which the carbon dioxid had been removed by passing it through wash bottles containing sodium hydroxid and calcium hydroxid solutions respectively. The tubes were examined as well as could be, without opening the jars, at the end of 7 days and again at the end of 10 days. Growth was observed on both occasions in tubes having reaction of—5 but in no others. At the end of 24 days the jars were opened and the tubes examined critically for growth. All those calculated to have a reaction of —5 showed growth, but the stronger concentrations were inhibitory in all cases. Check tubes calculated to have a reaction of —5 were titrated and were found to react at from —3 to —5 Fuller's scale.

Optimum reaction.—As a basis for determining optimum reaction, nutrient bouillon having a reaction of 0 Fuller's scale was employed. Portions of 100 cc. each were treated with the requisite amount of hydrochloric acid or sodium hydroxid to give reactions at intervals of 5 points of Fuller's scale. Material was tubed, sterilized, and inoculated with a 2 mm. loop of broth cultures, and placed in the incubator at 25°. The most vigorous early growth occurred in tubes having reactions of +10 and

+15 Fuller's scale. The general appearance of the cultures as regards turbidity, pellicle formation and rate of development indicated that the optimum reaction is very close to +10 Fuller's scale.

Temperature relations.—The optimum temperature was determined in nutrient broth having reaction of +10 Fuller's scale. Cultures were incubated at 20, 25, 30 and 37° C. The best growth was obtained at 25° C. The maximum temperature was not much above 37° C. and only very feeble transient growth was obtained at body heat. No attempt was made to determine the minimum temperature, but growth was slow at 10° C.

Thermal death point determinations were made upon a very large number of cultures. For this work thin walled test tubes as uniform as possible and containing 10 cc. of nutrient broth were used. Inoculations were made from 18 to 24 hour old broth cultures. Great care was taken to avoid slopping the unheated inoculated broth upon the sides of the tubes above the level of the medium. Within an hour after inoculation the tubes were placed in a water bath so that the broth was well below the surface of the bath, and heated for exactly 10 minutes. The temperature was kept constant within .1° C. by a sensitive temperature regulator and a mechanical agitator. After removal from the bath the tubes were cooled in air and placed in the incubator at 25° C. The results of thermal death point determinations were not absolutely uniform but in nearly every case the death point was found to fall between 49 and 50° C. A few tubes heated at 50° showed growth, and in some cases growth occurred after heating at a fraction of a degree above 50. In a large majority of the cases the death point fell between 49.5 and 50° C. In no case did death result from heating for 10 minutes at less than 48.9° C.

Growth in carbon dioxid atmosphere.—Tubes of nutrient broth were heated to drive out the dissolved oxygen, cooled by dipping in cold water, quickly inoculated, and placed with con-

trols in a Novy jar, the cover of which was sealed with Darwin's wax mixture. Carbon dioxid prepared in a Kipp generator from boiled marble chips and chemically pure hydrochloric acid was passed successively through wash bottles containing 10% sodium carbonate, 10% potassium permanganate and boiled distilled water respectively, and then through the Novy jar. The cotton plugs employed were as loose as was consistent with the safety of the cultures and the jar was exhausted with the vacuum apparatus several times to aid in removing all oxygen. After carbon dioxid had passed through the jar two hours and a half it was sealed and incubated at room temperature. After 20 days the jar was opened and the tubes examined for growth with negative results. Within 24 hours after air was admitted to the culture all except the controls developed characteristic growth.

A second series of cultures was prepared in the same manner as the first except that a 2% dextrose nutrient broth was employed. Five days after inoculation fermentation became apparent in several tubes, as could be seen from the gas collecting in bubbles at the surface. After two weeks all inoculated tubes showed fair growth. Pellicle and ring formation were absent. The growth was characterized by a tendency to granular formation throughout the medium and the evolution of appreciable amounts of gas on agitation. When air was admitted to the cultures moderate clouding promptly developed and the usual aerobic characters appeared.

Desiccation.—For desiccation tests small portions of 24 hour old broth cultures were transferred to sterile cover slips in sterile Petri dishes. Only a very small amount of material was transferred and this was spread in a thin film on the cover slip. The preparations were allowed to dry at room temperature, 20 to 24°. Slips were transferred to tubes of nutrient broth with sterile forceps as soon as dry, and at the end of 3 hours, 17 hours, 24 hours, 41 hours, 50 hours, 6 days, 9 days, and 20 days. In no case was the organism killed by less than

6 days drying. In one of the 5 tests made no growth was obtained in a tube inoculated with a cover slip dried for 6 days. Growth occurred 2 days after inoculation with cover slips dried for 9 days. No growth occurred in tubes inoculated with cover slips dried for 20 days. Tubes were held under observation for 10 days after the cover slips were introduced.

Insolation.—Agar plates were sown with such dilutions as to produce from 100 to 200 colonies, and allowed to stand until thoroughly hardened. One-half of the plate was covered with an opaque screen, and the cultures exposed on ice to the influence of direct sunlight for 15 minutes. The exposure was made in the middle of the day on August 28. After exposure the plates were returned to the incubator and allowed to remain at a temperature of 25° for 3 days. The colonies were then counted upon exposed and unexposed portions of the plate, with results as follows:

	Exposed side	Unexposed side
	81 colonies	117 colonies
	65 "	85 "
	58 "	80 "
	16 "	27 "
	20 "	50 "
	5 "	85 "
	13 "	40 "
Total.	258 "	484 "
Difference,	226 "	Calculated percent killed, 46.7

Production of alcohol.—Four different media were employed in the determination of alcohol production, viz.: 2% dextrose bouillon, 2% sucrose bouillon, potato broth, and maple sap. Inoculation was made in liter flasks in 500 cc. portions of media to which 10 gr. of calcium carbonate had been added immediately before sterilization. The cultures were held at room temperature, 20 to 24° C. After 10 days incubation the undissolved calcium carbonate was removed by filtration, the cultures made slightly acid with hydrochloric acid and then distinctly alkaline with sodium carbonate, the precipitated calcium car-

bonate removed, the filtrate distilled, and about 75 cc. of the distillate collected. A portion of the distillate was tested for alcohol and allied bodies by adding 5 to 6 drops of a 10% solution of potassium hydroxid, bringing the solution to a temperature of 50° C., and, then, adding a saturated solution of iodin in potassium iodid drop by drop until the liquid had a permanent brown color. After thorough agitation, the potassium hydroxid solution was added drop by drop until the color disappeared. In every instance a copious formation of iodoform resulted. None of the controls gave the precipitate. This reaction is not peculiar to alcohol, but is produced also by acetone, aldehyde, isopropyl alcohol, propylic and butylic alcohols and aldehydes, various ethers, meconic, laevulic, and lactic acids, turpentine, sugar, etc. On the other hand it is not given by methyl or amyl alcohol, chloroform, chloral, glycerin or ether; nor by acetic, formic or oxalic acids.[1] In general substances containing the group CH_3C linked to oxygen answer to the iodoform test.[2]

The iodoform producing bodies likely to be present in this distillate were alcohol or bodies belonging to the aldehyde or ketone groups. In order to determine if possible whether the body present was an alcohol, aldehyde or ketone, or whether all three were present, portions of the distillate were subjected to the following tests:

1. A few drops of 10 percent sodium hydroxid were added to a few cc. No distinctive reaction occurred, but upon standing a small amount of slimy or slightly resinous precipitate collected at the bottom of the tube. This was present, however, in only minute amounts.

2. A solution of Merck's phenol dissolved in excess of sulphuric acid when treated with a few cc. of the solution gave no characteristic scarlet color.

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[1] Allen. Commercial Organic Analysis, 1, p. 91 (1903).
[2] Holleman, A. F. Textbook of Organic Chemistry, p. 173 (1903).

3. Hot Fehling's solution in excess was reduced by a portion of the distillate, giving characteristic copper precipitate.

4. A saturated cooled solution of acid sodium sulphite treated with a portion of the distillate gave no reaction.

5. The color of a solution of fuchsin, mixed with just sufficient sodium sulphite almost to decolorize it, was not restored by the addition of 5 cc. of the distillate, although in some cases there appeared to be a slight suggestion of returning color.

6. A 10% solution of silver nitrate was treated with an equal quantity of 10% solution of potassium hydroxid and ammonia was added until the precipitate just dissolved. A portion of the distillate added to this reagent gave a metallic mirror after standing for half an hour, or within one or two minutes when the tube containing the mixture was gently heated over a flame.

The indications were that while aldehyde or ketone or both might be present in small quantities, the greater part of the iodoform must have been produced from alcohol. As a further check upon this conclusion 50 cc. of the distillate producing iodoform were added to an excess of hot Fehling's solution and the containing flask immediately connected with a condenser and distilled. About 20 cc. of the distillate were collected. An examination of the residue in the Fehling's solution showed a reduction of copper. The distillate gave a copious precipitation of iodoform, but failed to react with ammoniacal silver nitrate or with decolorized fuchsin.

Production of proteolytic enzyms.—The slow liquefaction of gelatin in old cultures and the suggestion of partial digestion observed in old milk cultures indicates that this organism produces proteolytic enzyms only when forced to do so and then only in very small quantities. In order to determine this point fresh centrifuged milk was passed through several thicknesses of fine meshed filter paper, and finally freed from the last traces of fat, and at the same time rendered sterile, by passing it

through a Pasteur filter. Portions of 50 cc. each were removed to sterile flasks by means of sterile pipettes and inoculated with the organism. The cultures were held at 25° C. and tested for proteolytic action at intervals from 10 to 44 days, always with negative results. The test was made by adding to the culture in the flask 75 grams of ammonium sulphate, heating in a water bath at 60° C. for 30 minutes to precipitate all proteids except peptones and propeptones, filtering, rendering the filtrate strongly alkaline with potassium hydroxid and testing with a few drops of a 1% solution of copper sulphate.[1]

Production of diastatic ferments.—A thin starch paste to which was added 2% of sugar free thymol was prepared and added to equal parts of 10 day old broth cultures. After incubation at 25° for 6 to 8 hours the solution was filtered and tested for reducing sugar with Fehling's solution. The results were negative.

Production of invertase.—A solution containing 2% of cane sugar and 2% of phenol was prepared for the purposes of this test. Equal quantities of 10 day old broth cultures of the organism and this solution were thoroughly mixed and left in the incubator over night. The unclarified solution when tested with Fehling's solution gave a pronounced change of color with a variable amount of copper precipitate, but the reaction was so far from characteristic that a check upon the work was desired. It is customary in analysis of solutions for invert sugars to clarify with some substance, such as neutral lead acetate or copper sulphate, before proceeding with the analysis. In the use of lead acetate it is important to avoid a large excess and it is necessary to remove the slight excess used by precipitation with calcium oxalate before proceeding with the Fehling test. In order to determine the influence of clarification of bacterial cultures before running the Fehling test the following solutions were prepared:

[1] Salkowski and Orndorff, loc. cit., pp. 15 and 46.

1. A solution containing 2% of phenol, 2% of sucrose, and .2% of dextrose.

2. A solution containing 2% of phenol, 2% of sucrose, and .2% of dextrose, together with an equal volume of sterile bouillon.

3. A solution containing 2% of phenol, 2% of sucrose, .2% of dextrose and an equal volume of a 10 day old broth culture of the organism.

4. A solution containing 2% of phenol, and 2% of sucrose

5. A solution containing 2% of phenol, and 2% of sucrose, together with an equal volume of sterile bouillon.

6. A solution containing 2% of phenol, 2% of sucrose, together with an equal volume of a 10 day old broth culture of the organism.

7. A solution containing 2% of phenol.

8. A solution containing 2% of phenol, together with an equal volume of sterile bouillon.

9. A solution containing 2% phenol, together with an equal volume of a 10 day old broth culture of the organism.

10. A solution containing sterile bouillon.

11. A solution containing a 10 day old broth culture of the organism.

Three 10 cc. portions A, B, and C of each solution were taken. To the solutions A, 1 cc. of a strong solution of lead acetate was added. They were filtered, the excess of lead acetate precipitated with potassium oxalate crystals, care being taken to avoid an excess of potassium oxalate. The lead oxalate was filtered off and the filtrate reserved for treatment with Fehling's solution. Portions B were treated with 1 cc. of copper sulphate solution used in connection with the Fehling's solution. Portions C were given no preliminary treatment before using the Fehling test. The precipitation was carried out in Erlenmeyer flasks. By means of a pipette 10 cc. of the copper solution was placed in the flask, then 10 cc. of the tartrate, care being taken to avoid gathering undissolved precipitate on the walls. The two were mixed and heated to boiling. A 10 cc. portion of the solution to be

tested was added to the mixture which was again brought to a boil and maintained at gentle ebullition for 2 minutes. It was then removed from the hot plate and transferred to a porcelain evaporating dish and left to settle for a few minutes. The liquid was decanted and the bottom of the dish examined for precipitated copper. The following table shows the results obtained. The presence of precipitated copper is indicated by the sign +, and its absence by the sign —. Where only a very minute precipitate was obtained it is indicated by the word "trace."

Prelim'y treatment	A Lead acetate	B Copper sulphate	C	Character of solution
1	+	+	+	phenol, sucrose, dextrose.
2	+	+	+	phenol, sucrose, dextrose, bouillon.
3	+	+	+	phenol, sucrose, dextrose, culture.
4	—	trace	trace	phenol, sucrose.
5	—	—	—	phenol, sucrose, bouillon.
6	trace	trace	+	phenol, sucrose, culture.
7	trace	trace	—	phenol.
8	—	—	—	phenol, bouillon.
9	—	—	—	phenol, culture.
10	—	—	—	bouillon.
11	—	—	—	culture.

The reaction in series A and B was in general characteristic. In series C, numbers 1 and 4 were characteristic, number 2 was nearly so, while numbers 3 and 6 gave a muddy solution with an uncharacteristic color and, in addition to the copper oxid, the precipitate contained a brown, light weight, flocculent material.

Cultures upon potato slants 4 days old were washed with distilled water, the washings were filtered and tested for reducing sugars with Fehling's solution with negative results.

Effect of germicides.—The germicidal effect of formaldehyde and of phenol were tested in broth cultures of the organism by adding definite known quantities of these germicides to tubes of sterile medium and then inoculating with a 2 mm. loop of young broth culture.

Formaldehyde.—As a basis for this work a guaranteed 40% formaldehyde was employed. One cc. of the solution was added

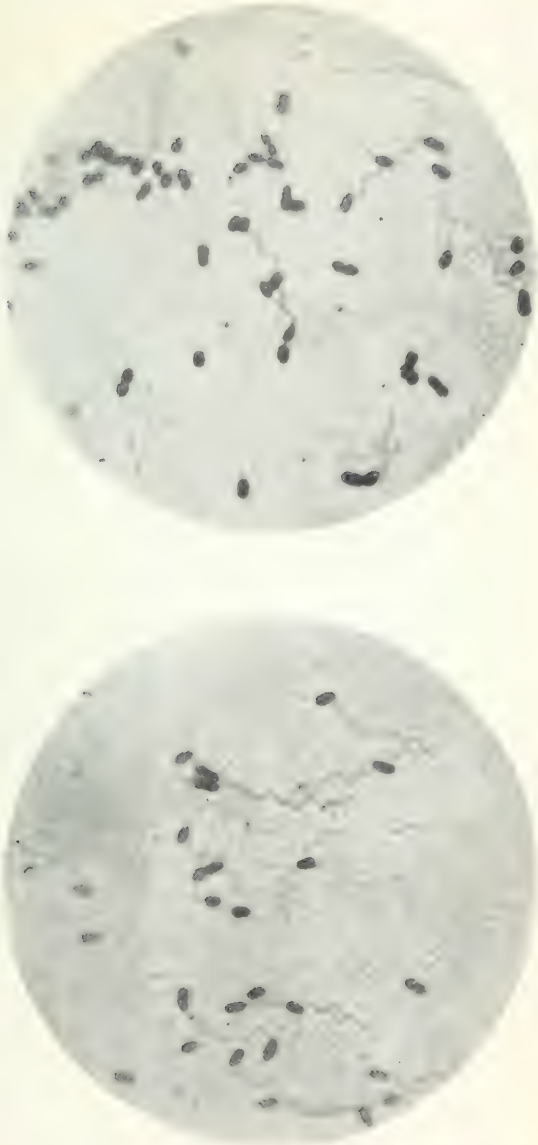

PLATE XI.—*Ps. fluorescens var. non-liquifaciens*, strain CXL. Flagella preparations from 24-hour agar slant, (Loeffler's stain). (See page 555.) × 1500.

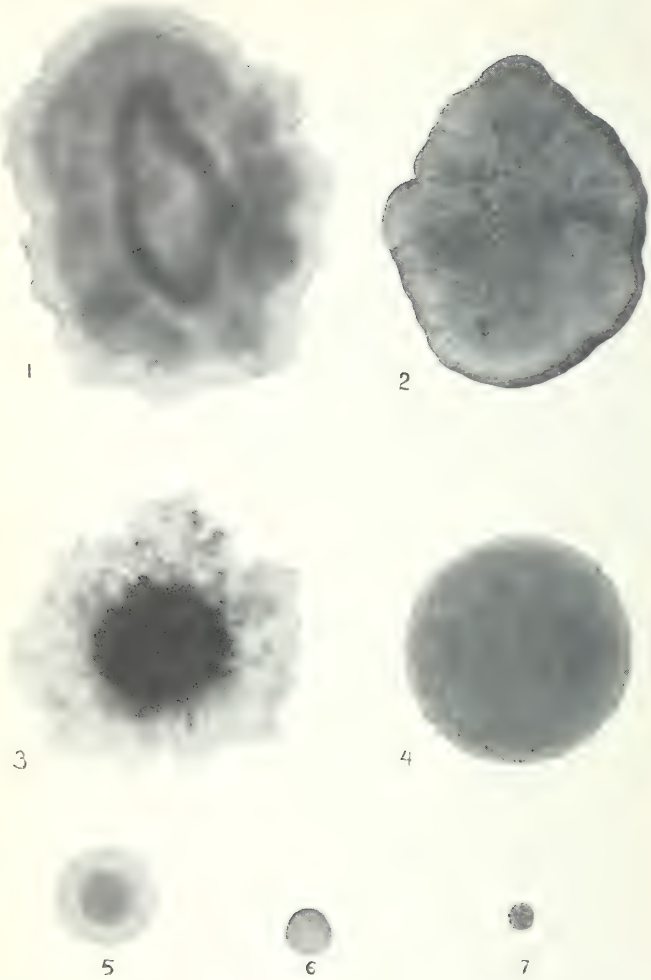

PLATE XII.—*Ps. fluorescens.* Types of gelatin colonies. Figures 1 and 2. Strain LIII (two day colony). Figures 3 and 4. Strains LV and 5 (two day colonies). Figures 5, 6 and 7. Strains XXXIII (two day colony) XXXVI and XXXIII (one day colonies). (See pages 567-568.) × 75.

to flasks containing 49, 99, 149 and 199 cc. respectively of distilled water. From each of these flasks portions of 1 cc. were withdrawn with sterile pipettes and placed in test tubes containing 10 cc. of broth, thus making dilutions of 1-550, 1-1100, 1-1650 and 1-2200 respectively. Eight tubes of each dilution were prepared, 5 tubes were inoculated with a 2 mm. loop of a 24 hour old broth culture, and 3 were retained as controls. Incubation was at 25° C. No growth had developed in any of the tubes at the end of 2 days. On the fourth day growth was observed in one of the five tubes containing 1 part of formalin to 2200 of water. Thirteen days after inoculation transfers were made into sterile broth. The transfers from the tube showing growth developed a characteristic appearance but those from the other tubes remained unchanged. In the second series 1 cc. of 40% formaldehyde was added to flasks containing 199, 249 and 299 cc. respectively of distilled water. One cc. portions were transferred with sterile pipettes to tubes containing 10 cc. of broth, thus making dilutions of approximately 1 part of formalin in 2200, 2750, and 3300 parts respectively. As in the previous instance 5 tubes were inoculated and 3 retained as checks. Within 24 hours growth appeared in 3 of the 5 tubes containing 1 part of formalin to 3300. The second day growth was evident in all of the tubes containing 1 part of formalin to 3300 and in one of the tubes containing 1 part to 2750. The cultures 6 days old showed growth in all tubes of the weakest dilution, in 3 of the 5 tubes containing 1 part formalin to 2750 of the medium, and a very slight growth in one of the tubes containing 1 to 2200. On the eleventh day the tubes had all cleared except one containing formalin in the proportion of 1 to 3300. This tube showed strong clouding.

Phenol.—The phenol employed in this work was Merck's phenol, U. S. P. VII. This was melted by placing the container in warm water. To a portion was added 11% of water by weight, thus obtaining a solution which remained liquid at ordinary temperatures. One cc. of this liquid phenol was added to 9, 49, 99, 149, and 199 cc. respectively of distilled water. One

cc. portions of these various dilutions were added with sterile pipettes to tubes containing 9 cc. of sterile broth, thus making dilutions of 1-100, 1-500, 1-1000, 1-1500, and 1-2000 of liquid phenol in broth. As in the case of the formalin 5 tubes were inoculated and 3 retained as checks. Growth was observed at the end of a day in all tubes containing 1 part liquid phenol to 2000 parts of broth, and in those containing 1 part to 1500, while four of the five tubes containing 1 part of liquid phenol to 1000 parts of broth, showed slight clouding. Good growth eventually developed in all tubes containing 1 part to 1000. No further change was observed in these tubes during the 2 weeks they were held under observation.

Nitrogen requirements.—The water used in the study of the nitrogen requirements of the organism was re-distilled with potassium permanganate, after which a third distillation employing sulphuric acid was carried out. Only chemicals of known purity were employed, except in the case of glycerin which, while of a high grade of purity, was not free from traces of nitrogen.

The following formulas were used:

A.	B.	C.
Sodium chlorid, 6 grams.	Sodium chlorid, 6 grams.	Sodium chlorid, 6 grams.
Calcium chlorid, .10 gram.	Calcium chlorid, .10 gram.	Calcium chlorid, .10 gram.
Magnesium sulphate, .35 gram.	Magnesium sulphate, .35 gram.	Magnesium sulphate, .35 gram.
Di-potassium phosphate, 2.2 grams.	Di-potassium phosphate, 2.2 grams.	Di-potassium phosphate, 2.2 grams.
Glycerin (Merck's blue label), 35 grams.	Mannit, 35 grams.	Dextrose, 35 grams.
Water, 1000 cc.	Water, 1000 cc.	Water, 1000 cc.

Each of these solutions, A., B., and C., were divided into five portions, one of which in each case was reserved as a control. To the other four were added 1% of asparagin, urea, ammonium chlorid, and potassium nitrate respectively. The media were tubed and sterilized in the autoclave for 15 minutes under 5 pounds of steam.

Series A. *Glycerin.*—As would be expected, growth developed in the control tubes of series A where glycerin was employed as a source of energy, since they were not nitrogen free. Growth became apparent the third day, but was slight and transient. The tubes containing potassium nitrate appeared exactly like the controls. The asparagin tubes developed growth in six days which increased, becoming very pronounced in two weeks. At no time did development occur in tubes containing urea or ammonium chlorid.

Series B. *Mannit.*—No growth occurred in the controls or in tubes containing potassium nitrate. Very feeble growth was observed with ammonium chlorid and with urea. The asparagin tubes showed growth the sixth day which became very pronounced. A deep milky color appeared, and a thick, tough, pellicle formation and heavy sediment developed.

Series C. *Dextrose.*—The controls gave no growth. Potassium nitrate, ammonium chlorid and urea all developed feeble growth, which was less transient in the case of ammonium chlorid than with potassium nitrate. Asparagin again gave a luxuriant growth after a period of delay. The development upon asparagin was better when dextrose was used than when either glycerin or mannit was employed. Moreover the organism seemed to be able to make slight development upon potassium nitrate in association with dextrose but not when mannit was substituted, and apparently not when glycerin was substituted. Urea and ammonium chlorid supported feeble growth with mannit and dextrose but not with urea. The results are summarized in the following table in which the sign — denotes no growth, + feeble growth, ‡ abundant growth after delay, and ? transient growth probably attributable to nitrogen derived from the glycerin.

TABLE 47. GROWTH ON NITROGENOUS MEDIA

	A. Glycerin	B. Mannit	C. Dextrose
Control,	?	—	—
Asparagin,	‡	‡	‡
Urea,	—	+	+
Ammonium chlorid,	—	+	+
Potassium nitrate,	?	—	+

NAME

A careful comparison of the cultural and biochemical characters of this organism with those of bacteria previously described would seem to justify its recognition as a new species. The name *Bacillus aceris* (new species) is therefore suggested for it.

B. BRIEF DESCRIPTION OF THE PINK COCCI OF MAPLE SAP[1]

Pink colonies were frequently found upon the plates poured from sour sap, particularly late in the season. Some of these were produced by yeasts or yeast-like organisms while others were composed of micrococci. Four strains were selected for further study from the cultures of pink micrococci isolated. These were CXXVI, CIV, XLIX, and CVI. A brief description of the organisms is given here for the purpose of record. All appear to belong to the type of *Micrococcus roseus*. They resemble each other closely and will be described as though identical, any variations in behavior from the general type being noted as they occur.

MORPHOLOGY

Vegetative cells.—The organisms are coccoid in form, occurring singly, in twos, fours, or irregular packets. Division is in two planes. The average diameter as determined on agar hanging block cultures was 1.2 microns. The smallest organisms observed measured .8 micron and the largest 2. microns. Cultures in nutrient broth had a tendency to be somewhat smaller and to occur singly or in twos.

Spores.—Evidences of spore formation were absent.

Flagella.—The organisms were non-motile and no indications of flagella were observed.

[1] The matter appearing on pages 516-521 is briefed from a thesis entitled "The Pink Micro-Organisms of Maple Sap," presented by N. R. Smith, B. S., of the class of 1911, in partial fulfillment of the requirements for the baccalaureate degree in the College of Agriculture of the University of Vermont.

Capsule.—There was a tendency to develop a slimy sediment in liquid cultures, but microscopic examination and staining methods failed to demonstrate a capsule.

Zooglea.—No evidence of zooglea masses was observed.

Involution forms.—Involution forms were not observed.

Staining reactions.—The organisms stained readily in 1-10 cold watery fuchsin, gentian violet, Loeffler's alkaline methylene blue, and carbol fuchsin. The method of Gram gave negative or sometimes doubtful results.

Cultural Characters

Agar stroke.—Growth was moderate becoming visible two days after inoculation. It was raised, regular, slimy, and tinged with pink. Iridescence developed after 10 days, the growth having spread to a width of 4 to 5 mm.

Agar stab.—Slight growth became visible after 24 hours. The second day a characteristic surface colony appeared. The growth in old cultures (6 to 8 weeks) became villous along the line of puncture. Colonies were first gray, and then became pink. When 7 days old they were from 2 to 4 mm. in diameter, raised, shiny, and pigmented. The edge was regular, thin and transparent, and the center opaque.

Two percent sucrose litmus agar.—Growth was confined to the surface, and characterized by the production of pink pigment. Acid was produced but the litmus was not reduced.

Two percent dextrose litmus agar.—Acid production became apparent the second day. Reduction of the litmus began after one week and progressed slowly until complete about the fourth week.

Two percent lactose litmus agar.—Growth was slight, acid formation and litmus reduction both being absent.

Five percent glycerin litmus agar.—The growth was slow and acid production absent. Feeble litmus reduction was noted on the fifth day, and thereafter progressed slowly, becoming complete after two weeks.

Potato.—Organisms failed to develop visible growth upon cooked potato slants.

Gelatin stab.—Growth was slow, moderate, at first beaded along the line of puncture, becoming filiform above. Slight liquefaction began on the twenty-fourth day and continued slowly, becoming stratiform in six weeks. The liquefied gelatin became pinkish, contained a compact pink sediment and was distinctly viscid.

Gelatin plate.—Colonies appeared the third day, and attained a diameter of 2 mm. in 10 days. They were convex with round entire edges. The pigment upon gelatin was similar to that upon agar except that it was usually of a darker hue.

Broth.—First evidences of growth consisted of a slight, compact, pinkish sediment appearing the second or third day, while clouding appeared in one week. The sediment was viscid on agitation.

Milk.—The first evidence of growth consisted of a deposition of pink sediment, becoming apparent within the first 10 days. In 3 weeks the medium was colored light pink while a watery appearance developed the fourth week. This appeared to be associated with feeble digestive action, although complete peptonization did not occur. Coagulation did not take place.

Litmus milk.—The organisms studied developed considerable variation upon this medium. CXXVI showed acid formation after 14 days, with coagulation 2 or 3 days later, promptly followed by the reduction of the litmus in the lower part of the tube. Partial digestion of the curd occurred. The first evidences of peptonization appeared during the third week and progressed slowly until about the ninth week, when about half of the total curd had become dissolved. CIV exhibited no change in the medium until after 6 weeks, when it turned alkaline and curdled. Digestion did not occur. XLIX became alkaline the third week and developed a soft curd 3 or 4 days later. Litmus reduction occurred after 4 weeks. Partial liquefaction of the coagulum was observed in about half of the cultures of this strain, but did not

occur in the remainder. CVI curdled milk after 5 weeks with reduction of litmus. The color was restored with an alkaline reaction in the upper half of the tube after 8 weeks. No digestion of casein was observed.

The variations upon litmus milk were so great that the work was repeated several times and the purity of the cultures carefully verified, but without change in the character of the reaction.

Cohn's solution.—A slow growth was noted which was characterized by the development of sediment which usually was granular and flaky, but sometimes slightly viscid.

Uschinsky solution.—The growth was slow, at first moderate in amount, becoming abundant after 5 weeks, with copious sediment. A portion of the culture immediately above the sediment exhibited clouding.

Growth in bouillon over chloroform.—There was no growth in bouillon tubes to which 2 or 3 drops of chloroform were added.

PHYSICAL AND BIOCHEMICAL FEATURES

Acid production.—Nutrient broths to which were added 2% of various sugars and 5% of glycerin respectively were tested for gas formation and acid production in fermentation tubes. In no case was there sufficient gas production to become apparent with the Smith tubes. Ten cc. portions were titrated in the usual way at the end of 1, 2, 4, 10, and 28 days. The reaction of duplicate checks was subtracted from the average reaction of inoculated duplicates to determine the average acid or alkali production. The results expressed in percent normal are shown in the following table.

TABLE 48. ACID PRODUCTION ON CARBOHYDRATE BROTHS

	1 day	2 days	4 days	10 days	28 days
		Dextrose			
CXXVI,	—.13	—.08	—.05	.11	.45
CIV,	—.07	—.03	—.03	.22	.38
XLIX,	—.10	—.03	.07	.10	.40
CVI,	.10	.09	.06	.08	.39
		Sucrose			
CXXVI,	.06	.12	.03	.14	.56
CIV,	.08	.08	.03	.32	.62
XLIX,	.02	.05	.06	.07	.30
CVI,	.05	.10	.04	.11	.68
		Lactose			
CXXVI,	—.09	—.05	—.11	—.08	.60
CIV,	—.04	—.08	.06	—.10	.04
XLIX,	—.06	—.10	.01	—.08	.02
CVI,	—.03	—.04	—.08	—.08	.42

	Glycerin	
	10 days	20 days
CXXVI,	—.05	.10
CIV,	.05	.00
XLIX,	.00	—.05
CVI,	—.24	.25

Nitrate reduction.—Cultures grown in nitrate broth were tested for nitrites at the end of 5, 10 and 15 days, with uniformly positive reaction. Other cultures were tested with Nessler's reagent for ammonia with negative results.

Hydrogen sulphid.—Cultures on nutrient broth were tested for hydrogen sulphid production by means of moist filter paper, bearing lead acetate, suspended from the plug. No evidences of this gas were observed.

Indol production.—Cultures on Dunham's solution and on peptone water were tested for indol production with negative results.

Temperature relations.—The optimum temperature was from 20 to 25° C. The maximum was slightly above 40° C. Growth occurred at 10° but not at 4° C.

Thermal death point.—Thermal death point determinations were made by exposing young transfers in thin wall test tubes at various temperatures, in a constant water bath for ten minutes

XLIX failed to grow in tubes exposed at 44° C. or above, CIV at 46° C., and CXXIV and CVI at 48° C.

Desiccation.—Sterile cover glasses were placed in Petri dishes and inoculated, each with a loop of media from a 12 day old broth culture, and allowed to dry for intervals varying from the first instant when the cover glasses were free from the film of moisture up to 30 days. Their viability was tested by transferring the cover slips to tubes of broth by means of sterile forceps. The cultures were held for two months but in every instance failed to show growth.

Insolation.—Thinly sown agar plates were exposed on snow to direct sunlight for 30 minutes with one-half covered. After a period of incubation the colonies which developed on the exposed and unexposed portions of the plates were counted. There was practically no difference in the number of colonies developing.

Pigment formation.—Pigment formation occurred only in the presence of oxygen and at ordinary temperatures. It failed entirely at 37° C. Upon solid media such as agar and gelatin each coccus had its peculiar tinge of pink. An attempt was made to refer these colors to the chart given by Winslow,[1] as follows:

CXXVI	=	Orange red	III & IV.
CIV	=	Orange red	IV.
XLIX	=	Orange yellow	VI.
CVI	=	Orange red	III & IV.

The pigment was insoluble in water, dilute sulphuric acid, xylol, cold or hot alcohol, and chloroform. In dilute nitric acid the growth was disintegrated and the pigment dissolved slightly.

C. THE GREEN FLUORESCENT BACTERIA OCCURRING IN MAPLE SAP

Introduction

Bacteriological literature contains descriptions of more than 50 species of bacteria which are capable of producing a green fluorescent pigment. Such bacteria were originally assigned to

[1] Winslow. Systematic Relationships of the Coccaceæ (1908).

various widely separated groups, the early writers recognizing the ability to form the fluorescing pigment as a valuable diagnostic character, but apparently failing to consider a possible phylogenetic relationship. In the light of the intimate relationship revealed by the more critical studies of the later investigators, all green fluorescent bacteria are now classified in the Pseudomonas group of Migula. Moreover it is now recognized that many of the described species are identical, while the characters of others grade into each other so imperceptibly that present bacteriological methods fail to demonstrate specific constant differences upon which differentiation may well be based.

The members of the group are widely distributed. They are almost universally found in the presence of putrefying organic material, and are abundant in soil, water, and air. They are recognized as one of the most common types of water bacteria. Schmelck (26:546) noted that the predominating organisms in glacial waters were of this sort, and Harrison (12) and Belli (2) observed *B. fluorescens liquefaciens* in hail. Jensen (13:613) states that the fluorescent organisms occur in butter, while Thöni (30:623) noted their presence in fifteen samples of lemonade which he analyzed. Griffon (11) claims that *B. fluorescens liquefaciens* and *B. fluorescens putrida* are capable of producing wet rots of certain vegetables. The former has been reported as the causal organism in a carrot rot, while tobacco anthracnose is attributed to *B. aeruginosus*, which according to Griffon, is synonymous with *B. fluorescens putrida*. Tomatoes grown under glass have also been attacked by a stem canker said to be due to *B. fluorescens*. Although the majority of fluorescent bacteria are harmless, certain species are reported as pathogenic to animals. It is therefore evident that the bacteria of this group are capable of existing under a variety of conditions.

Fluorescent bacteria play a leading role in the deterioration of maple sap. Fresh drawn sap of the early "runs" is water clear and has a clean sweet flavor, but with the advent of warmer weather it becomes more or less cloudy and a disagreeable flavor

develops. Such sap is popularly called sour, and several type are recognized among which the most common is the so called green sap. In all the samples of greenish or greenish brown sap examined, the predominating organisms were of the fluorescent type, and inoculation experiments have repeatedly demonstrated their causal relationship to this type of spoiled sap.

The initial infection of the sap is doubtless brought in from the bark of the tree, by wind, and by dripping rain and snow water. Falling snow and rain also probably bring in some infection from the air. Fluorescent bacteria were demonstrated on the tree bark as well as in the snow and surface water and in unwashed buckets and spouts from the previous season. Large numbers of the organisms remain in the spout and buckets from season to season, unless these are thoroughly cleansed by boiling.

Such maple sap organisms of the fluorescent type as have been previously reported upon (4:492) were recognized as members of the Pseudomonas group of Migula and more or less closely related to *Pseudomonas fluorescens*. Both the liquefying and non-liquefying types were observed.

On account of the prevalence of this type of bacteria in maple sap and their importance in the maple sirup industry, a critical study of the characters of the group was considered important. The discussion falls naturally under two heads:

(1) A preliminary investigation with 42 strains of green fluorescent sap bacteria.

(2) A more exhaustive comparative study of seven representative strains selected in the preliminary work and of six known species.

PRELIMINARY STUDIES UPON 42 STRAINS OF GREEN FLUORESCENT SAP BACTERIA

ISOLATION

The 42 strains of green fluorescent sap bacteria were selected from several hundred cultures isolated from abnormal maple sap secured in different parts of Vermont during four successive

sugar seasons. The numbers of the strains selected, the dates of isolation and the sources are as follows:

Strain	Date of isolation		Source
CXII,	4/4/09	Randolph.	Sour tree, Orchard 1
CXV[1],	"	"	Second sour tree, Orchard I.
CXXVII,	4/5/09	"	Third sour tree, Orchard I.
CXXVIII,	4/12/09	"	Green sour sap employed in making sirup 24 (p. 358).
CXXIX,	"	"	
CXXX,	"	"	
CXXXIII,	4/12/09	"	Green sap, Orchard II.
CXXXIV,	"	"	
CXXXV,	"	"	
CXXXVII,	"	"	
CXXXVIII,	"	"	
CXXXIX,	"	"	
CXL,	4/12/09	"	Milky sap, various orchards.
CXLI,	"	"	
CXLII,	"	"	
CXLIII,	"	"	
CXLV,	"	"	
CXLVI,	"	"	
CXLVII,	"	"	
CXLVIII,	4/13/09	"	Greenish-yellow sour sap from same tree as sirup 26 (page 358).
CXLIX,	"	"	
CL,	"	"	
CLI,	"	"	
CLII,	"	"	
CLIII,	"	"	
CLIV,	"	"	
CLV,	"	"	
CLVI,	"	"	
CLVIII,	"	"	
CLIX,	"	"	
CLXXVII,	"	"	
CLXXIX,	"	"	
5	4/27/07	E. Montpelier	Sour sap.
XVI,	2/1/08	Burlington	Sour sap.
XXXIII,	4/11/08	Bethel	Sour sap.
XXXVI,	"	"	
XXXVIII,	"	"	
L,	4/18/08	Fairfax	Sour sap, Orchard I.
LI,	"	"	
LIII,	"	"	
LIV,	4/18/08	"	Sour sap, Orchard II.
LVI,	"	"	

[1] Doubtfully fluorescent.

METHODS OF WORK

Throughout the studies great care was exercised to secure uniformity of methods so that the results with the several strains might be comparable. In general the methods recommended in "Standard Methods of Water Analysis" (1905) and in Smith's "Bacteria in Relation to Plant Diseases" (27) have been followed, but in certain respects it has been deemed wise to introduce variations. The most important of these is the substitution of "Liebig's Extract of Meat" for beef infusion. Media prepared with meat extract has been used in this laboratory for several years and has always given satisfaction. It is believed that media prepared in this way is more uniform in composition than that from infusion and it also possesses the very decided advantage of being free from muscle sugar. Actively fermenting strains of *B. coli* develop in it without producing visible gas. The objections usually advanced against meat extract that it frequently contains resistant spores, that it may have been treated with preservatives, or that it may contain injurious by-products of bacterial development, have not seemed to be well founded. In our experience there is never more difficulty in sterilizing meat extract than in sterilizing beef infusion and we have never been able to discover indications of preservatives or injurious decomposition products, the most sensitive organisms developing as readily upon one type of medium as upon the other. When cultures are to be employed in studying gas evolution or acid formation, muscle sugar introduces a complication. It is the general custom to remove this by cultivating an active fermenter in it for a few hours. This is objectionable and is certain to introduce decomposition products to such an extent as to render the media quite unfit for the development of certain sensitive species. There is great need of synthetic substitutes for the beef media composed of synthesized chemicals, but until they are developed we believe the employment of a standard brand of meat extract has points of advantage over infusion.

The details necessary for duplication of our media are given below for the convenience of any one who may have occasion to review the results. Unless otherwise noted, media were prepared with a distilled water of high purity and adjusted against phenolphthalein to a reaction of +10 Fuller's scale. Sterilization was effected by exposing in the autoclave at 6 pounds pressure for 15 minutes, except with milk, potato, gelatin, and carbohydrate and glycerin media which were steamed in the Arnold for 15 minutes on each of three successive days.

Nutrient broth.—Ten grams of "Witte's Peptonum Siccum" and 5 grams of "Liebig's Extract of Meat" were added to one liter of distilled water and cooked in a double cooker until dissolved (about 30 minutes), restored to volume, titrated and adjusted in reaction to +10 Fuller's scale, cooked in double cooker 20 to 30 minutes, boiled 5 minutes over the open flame, titrated and adjusted in reaction if necessary, filtered and sterilized.

Agar medium.—This medium was prepared by adding 1.5% of Bausch and Lomb agar flour to the nutrient broth, with subsequent autoclaving, adjusting of reaction to +10 Fuller's scale, and clearing with either fresh white of egg or dried albumen.

Gelatin medium.—Nutrient broth was stiffened with 12% of "Nelson's Photographic Gelatin No. 1" unless otherwise specified. The broth was heated, the gelatin added, steamed a few minutes in the Arnold, adjusted to +10 Fuller's scale, cooled, cleared with egg albumen, filtered, and sterilized by the intermittent method.

REJUVENATION AND STOCK

The organisms were rejuvenated as recommended by transferring to nutrient broth, incubating 24 hours, transferring from the young culture to a second tube of broth, incubating 24 hours again, and so on for three days. Plates were poured from the third tube of broth to insure the purity of the strain and transfers from the colonies were made to tubes of agar and broth. The agar and broth series was transferred monthly

and from it, as a parent stock, a sub stock was maintained. From the sub stock an inoculation stock was rejuvenated occasionally and subjected to retransfer every 24 to 48 hours. Frequent replating assured the purity of the strains. Unless otherwise stated inoculations were made from 24 to 48 hour broth culture and incubation was at 25° C. Gelatin media were incubated at 20° C.

DETAILED FEATURES OF THE FORTY-TWO STRAINS

The immediate object of the preliminary studies was to separate the forty-two strains of fluorescent bacteria into groups, in order that type organisms might be selected for a more exhaustive study. To this end attention was directed primarily toward the characteristics which are expressed by the group number, (Descriptive Card, Society of American Bacteriologists); but considerable additional data was secured on the morphological, cultural, physical and biochemical characters of the organisms. Therefore the discussion of the preliminary work follows in a general way the detailed features as outlined in the Society Card. For the group numbers of the strains and the method of separating the series into groups, see pages 550-551.

I. MORPHOLOGY

1. *Form.*—The organisms are all motile rods. Preparations from 24 hour agar slant cultures of all strains stained by Löwit's method for flagella showed that some strains typically have one, and others three or six, polar flagella, chains frequently exhibiting numerous long flagella in a polar tuft. Strain CXV also has lateral flagella (c. f., pp. 546, 555).

2. *Grouping.*—Short chains of two cells were typical but longer chains were not uncommon. A surface scum or pellicle was usually present on liquid media.

3. *Motility.*—Rapidly and quite persistently motile.

4. *Endospores.*—None were demonstrated with stains or by thermal death point determinations. Several common spore

stains were applied on material from many media and, except for polar bodies, were without result.

5. *Capsules.*—Broth cultures of the three strains, XXXIII, CXV, and CXLV, were commonly stringy. Capsules were demonstrated on these strains, both by Welch's method and by Richard Muir's contrast stain. Attempts to demonstrate capsulation on some of the other strains were unsuccessful.

6. *Stains.*—All strains, except CXV, were Gram negative, though decolorization was not always complete, granulations in the rods and polar bodies being common. Strain CXV retained Gram's stain after 4 minutes in absolute alcohol.

Aqueous anilin stains.—Cells of all strains were easily stained, but gave up the dye readily on washing. The rods often showed granulation or a bi-polar effect, certain portions seeming to have a greater affinity for the stain than others.

II. Cultural Features

1. *Agar stroke.*—Growth abundant, filiform to echinulate, spreading below; raised, smooth, glistening and somewhat viscid or slimy. Medium more or less green fluorescent with all strains. Strain CXV showed at most only a doubtful fluorescence.

2. *Potato.*—Growth moderate with all strains. A rather narrow, smooth, filiform, light brown streak, becoming thick and spreading later. Medium sometimes greened but not invariably. Strain CXV often showed a dull rhizoid growth.

3. *Agar stab.*—Best development at top of puncture but considerable sub-surface growth later. Filiform, becoming villous. Varying degrees of green fluorescence. Long crystals were common in old cultures; they occurred under the surface growth and about the puncture and were never detected in control tubes. Similar crystals are illustrated by Smith (27:66). Beautiful green fluorescence, varying somewhat in intensity with the various strains. Strain CXV was doubtfully fluorescent.

4. *Gelatin.*—Nutrient broth, stiffened with 10, 12, and 15% gelatin, was used. Several complete sets of the strains were

tested out at 20° C. with similar results. The growth was best at the top with the puncture beaded to filiform. Restricted development occurred along the puncture below the liquid gelatin. Crateriform liquefaction becoming stratiform was typical of the group. The reaction began in from 16 hours to 3 to 5 months. Several weeks or longer was required to complete the liquefaction of 7 cc. of the medium. Fluorescence was not marked, old cultures showing it only faintly.

One set of cultures in duplicate held under observation over eight months yielded noteworthy results. Seven strains began a typical liquefaction after from three to five months, but the time required for complete liquefaction of 7 cc. was not determined. At the end of 8½ months control plates were poured and the organisms found to be present in pure culture. The organisms recovered were fluorescent and capable of producing hydrogen sulphid, the latter property being peculiar to these seven strains.

5. *Nutrient broth.*—A surface scum or a thin membranous pellicle, often wrinkled, was usually present. Clouding was strong at first, the medium becoming yellow-green fluorescent and clearing with a deposition of a more or less coherent sediment.

6. *Milk.*—Fresh centrifuged milk reacting +12 to +14 Fuller's scale, and sterilized by the fractional method, was used. In from one to ten days a watery layer appeared at the surface with a jelly-like thickening and greenish discoloration of the substratum in all cultures except those of the following strains: CXL, CXLI, CXLII, CXLIII, CXLVI, CXLVII, XVI, XXXVI, L, LI, and LIV. After 35 days' incubation, CXLVII, XVI, XXXVI, XXXVIII, LI, and LIV showed doubtful clearing. Strains CXL, CXLI, CXLII, CXLIII, CXLVI, and L in all trials remained unchanged for about 3 months, when a very slow digestion appeared.

7. *Litmus milk.*—Litmus milk was prepared by the addition of 2% of a saturated solution of chemically pure litmus to fresh centrifuged milk and which was then sterilized by the intermittent method.

Without enumerating the details of the minor differences observed with the several strains the characteristics on this medium may be summarized as follows: Alkali production and more or less complete reduction of litmus occurred with all strains. With the exception of strains CXL, CXLI, CXLII, CXLIII, CXLVI, XVI, XXXIII, XXXVIII, L, LI, and LIV, alkali was produced promptly in the surface layer which soon cleared, changed to acid and became more or less green in color. The alkali layer diffused slowly downward, several well defined strata often being differentiated. Digestion proceeded slowly with the enumerated strains, and the medium gradually became more and more blue, changing to purple at the time digestion became evident.

8. *Gelatin colonies.*—A rather superficial examination of the colonies of all strains on gelatin showed that they were very much alike. Liquefaction occurred quickly in all strains except CXL, CXLI, CXLII, CXLIII, CXLVI, CXLVII, XVI, XXXIII, L, LI, and LIV. For complete description of the types of colonies, see detailed characteristics of individual strains, pages 567-568.

9. *Agar colonies.*—There were no essential differences between the agar colonies of the several strains. For description of types, see detailed characteristics of individual strains pages 568-570.

10. *Cohn's nutrient solution.*—This medium was made according to the following formula:

Distilled water,	1000. cc.
Di-potassium phosphate,	5. gr.
Magnesium sulphate,	5. "
Ammonium tartrate,	10. "
Potassium chlorid,	0.5 "

All strains were tried on this medium several times, but only two strains, LI and LIV, showed growth. These grew well with fluorescence, and the growth was accompanied by the formation of crystals of magnesium ammonium phosphate. The development was gradual but persistent, fluorescence and crystals

appearing in from 15 to 23 days. Those strains showing no growth were transferred to broth after twenty days. The transfers developed normally becoming fluorescent in two days.

11. *Uschinsky solution.*—The medium was prepared as follows:

Distilled water,	1000. cc.
Glycerin (Merck's Blue Label),	40. "
Sodium chlorid,	5. gr.
Calcium chlorid,	0.1 "
Magnesium sulphate,	0.4 "
Di-potassium phosphate,	2.2 "
Sodium asparaginate,	4. "

All strains except CXV developed well on this medium, showing strong fluorescence, and a viscid pellicle and sediment. Transfers made from tubes of CXV on Uschinsky solution developed well in broth.

12. *Nitrogen requirements.*—For determining the nitrogen requirements, a modified Uschinsky solution prepared as follows was used as a basal medium.

Distilled water,	1000. cc.
Glycerin (Merck's Blue Label),	35. "
Sodium chlorid,	6. gr.
Calcium chlorid,	0.1 "
Magnesium sulphate,	0.35 "
Di-potassium phosphate,	2. "

The mixture was warmed to secure complete solution and divided into three portions, one of which was reserved as a control while the others were treated respectively with 0.5% of c. p. urea and 1% of asparagin.

No growth, or only a slight trace, occurred in the control medium. The feeble trace of transient growth in a portion of the controls was attributed to the traces of nitrogen known to be present in the glycerin.

Upon the urea medium all showed good growth, except strain CXV which failed to develop. Transfers of this strain from the urea medium to broth developed promptly.

Eight strains developed with fluorescence upon the asparagin medium. These were as follows: CXXIX, CXXXIII, CXLVIII, CXLIX, CLIX, CLXXVII, 5 and LVI. Transfers to nutrient broth from the asparagin cultures of those strains which failed to grow developed promptly.

III. Physical and Biochemical Features

1. *The action of the organisms upon dextrose, lactose, sucrose and glycerin.*—The carbohydrate and glycerin media were made by adding 2% of the sugars and 5% of glycerin respectively to bouillon which was sugar free as shown by the *B. coli* test. Cultures were observed for acid and gas production and for growth in the closed arm of the Smith tube.

Cultures and controls were analysed in duplicate for acid production after 1, 2, 4, 10, and 20 days incubation. Five cc. of the material were pipetted into 45 cc. of distilled water in Erlenmeyer flasks; ½ cc. of 1% phenolphthalein solution was added and the mixture boiled 2 minutes and titrated hot with N/20 sodium hydroxid. Boiling often increased the amount of free acid, probably through the volatilization of ammonia formed simultaneously with the acid.

Control titrations were made upon bouillon cultures. Some acid was detected which is thought to have resulted from the decomposition of proteids, since muscle sugar was absent. The conclusions as to acid production which are indicated in the group number, pages 548 and 550, were drawn after comparing the results on the carbohydrate media with those on inoculated bouillon.

No gas was produced from the sugars or glycerin. Growth in the closed arms of the fermentation tubes was limited and occurred only in old cultures.

The detailed discussion of the characters on various carbohydrates follows:

Dextrose.—Acid production without gas evolution was characteristic. Two % dextrose litmus agar developed an acid

reaction in from 18 hours to 4 days. Two % dextrose bouillon in fermentation tubes, titrated hot with N 20 sodium hydroxid against phenolphthalein showed acid production, considerably more being present than in sugar free bouillon. (See tables 49, 50, 51). Previous to boiling, the acid was masked by the ammonia produced, the cultures became pink on addition of phenolphthalein but decolorized on boiling, with increased acidity.

Lactose.—Litmus milk showed an alkaline reaction, becoming doubtfully acid after several weeks. No acid, or a trace in a few cases, was found by titrating 2% lactose bouillon. (Tables 52, 53). By comparison most cultures in lactose bouillon were found to produce no more acid than the control cultures in sugar free bouillon.

Sucrose—Same as lactose (table 54)

Glycerin.—Acid production without gas formation was observed with 11 strains on bouillon containing 5% of "Merck's Blue Label" glycerin. (See tables 55, 56).

The figures in the tables represent the percent of normal acid or alkali produced in the respective media and were calculated by subtracting the average reaction of duplicate controls from the average reaction of duplicate cultures, or vice versa.

TABLE 49. ACID AND ALKALI PRODUCED IN SUGAR FREE NUTRIENT BOUILLON
Test tubes, 10 cc.

Strain number	3 days		10 days	
	Acid	Alkali	Acid	Alkali
CXII,		0.30	0.06	
CXV,		0.09		
CXXVII,		0.16	0.04	
CXXVIII,	0.05		0.28	
CXXIX,	0.10		0.42	
CXXX,		0.02	0.26	
CXXXIII,			0.21	
CXXXIV,		0.21	0.15	
CXXXV,		0.36		0.22
CXXXVII,		0.31		0.12
CXXXVIII,		0.29		0.16
CXXXIX,		0.18		0.27
CXL,		0.35	0.36	
CXLI,		0.29		0.49
CXLII,		0.43		0.50
CXLIII,		0.25		0.62
CXLV,		0.03		0.94
CXLVI,		0.30	0.30	
CXLVII,		0.17		0.79
CXLVIII,	0.20			0.41
CXLIX,		0.21	0.48	
CL,		0.20		
CLI,		0.32		0.25
CLII,		0.35		0.49
CLIII,	0.03			0.55
CLIV,		0.14	0.16	
CLV,		0.14	0.07	
CLVI,		0.18		0.09
CLVIII,		0.13	0.34	
CLIX,		0.28	0.21	
CLXXVII,		0.33	0.03	
CLXXIX,		0.14		0.22
5,		0.18		0.25
XVI,		0.43		0.04
XXXIII,		0.14		0.51
XXXVI,		0.51		0.68
XXXVIII,		0.03		0.47
L,		0.36		0.36
LI,		0.16		0.59
LIII,	0.44			0.72
LIV,		0.11		0.11
LVI,		0.01		0.74

TABLE 50. ACID AND ALKALI PRODUCED IN 2% DEXTROSE BOUILLON

Fermentation tubes

Strain number	1 day		2 days		4 days		10 days		20 days	
	Acid	Alk.	Acid	Alk.	Acid	Alk.	Acid	Alk.	Acid	Alk.
CXII,	1.36		2.15		2.36		2.20		1.50	
CXV,	0.00		0.48		0.59		0.48		0.70	
CXXVII,	1.16		1.53		1.44		1.20		1.10	
CXXVIII,	0.53		1.08		0.92		0.84		0.71	
CXXIX,	1.00		1.88		1.90		1.66		2.13	
CXXX,	0.78		0.92		0.96		0.65		1.19	
CXXXIII,	0.00		0.63		0.76		0.60		0.80	
CXXXIV,	0.00		0.56		0.60		0.08		0.13	
CXXXV,	0.38		0.32		0.28		1.72		0.63	
CXXXVII,	0.24		0.28		0.79		2.10		1.01	
CXXXVIII,		0.06	0.00		0.10		1.19		0.08	
CXXXIX,	0.48		0.51		0.43		1.72		0.62	
CXL,	1.21		1.40		1.42		2.63		1.38	
CXLI,	1.06		1.13		1.02		2.34		1.04	
CXLII,	1.01		1.20		0.97		2.27		1.13	
CXLIII,	1.31		1.39		1.55		2.67		1.35	
CXLV,	0.66		1.44		1.30		2.55		1.41	
CXLVI,	0.80		0.99		1.04		1.13		1.01	
CXLVII,	0.57		0.66		0.56		0.83		0.87	
CXLVIII,	0.73		1.28		1.41		1.55		1.89	
CXLIX,		0.11	0.35		1.24		2.08		2.31	
CL,	0.81		1.13		1.14		1.25		1.17	
CLI,	0.42		0.45		0.44		0.33		0.79	
CLII,	0.46		0.27		0.28		0.29		0.27	
CLIII,	0.22		0.46		0.94		1.25		1.13	
CLIV,	0.32		0.40		0.78		0.73		1.33	
CLV,	0.30				0.52		0.93		0.20	
CLVI,	0.30				0.38		0.60		0.40	
CLVIII,	0.35				0.60		1.07		0.55	
CLIX,	0.21				0.82		0.98			0.12
CLXXVII,	0.36				0.60		1.03		2.65	
CLXXIX,	0.34				0.65		0.91		1.10	
5	0.71				0.96		1.06		1.90	
XVI,	0.70				0.78		0.68		1.58	
XXXIII,	0.37				0.94		0.83		1.72	
XXXVI,	1.28				1.64		1.51		2.12	
XXXVIII,	0.05				0.70		0.71		1.66	
L,	0.31				0.40		0.39		1.10	
LI,		0.04			0.50		0.49		1.28	
LIII,	0.23				0.55		1.11		0.62	
LIV,	0.00				0.58		0.51		1.68	
LVI,	0.61				0.90		1.10		1.65	

TABLE 51. ACID AND ALKALI PRODUCED IN 2% DEXTROSE BOUILLON
Test tubes, 10 cc.

Strain number	1 day		2 days		4 days		10 days		20 days	
	Acid	Alk.	Acid	Alk.	Acid	Alk.	Acid	Alk.	Acid	Alk.
CXII,	0.41		0.44		0.89		1.76		3.13	
CXV,	0.03		0.21		0.28		0.71		0.75	
CXXVII,	0.41		0.46		0.71		1.06		0.22	
CXXVIII,	0.43		0.78		1.05		1.22		1.24	
CXXIX,	0.48		0.55		1.54		2.23		2.50	
CXXX,	0.41		0.80		1.15		1.44		1.48	
CXXXIII,	0.10		0.17		0.37		0.85		1.06	
CXXXIV,	0.07		0.38		0.69		0.80		0.77	
CXXXV,	0.17		0.18		0.23		0.36		0.99	
CXXXVII,	0.10		0.16		0.29		1.08		1.16	
CXXXVIII,	0.03		0.16		0.43		0.54		0.94	
CXXXIX,	0.23		0.19		0.30		0.48		0.50	
CXL,	0.87		1.06		1.66		1.52		1.61	
CXLI,	0.58		0.81		1.13		1.35		1.71	
CXLII,	0.34		0.91		1.24		1.30		1.51	
CXLIII,	0.93		1.47		1.60		1.62		1.53	
CXLV,	0.24		0.51		0.54		0.73		1.35	
CXLVI,	0.39		0.64		1.02		1.40		1.55	
CXLVII,	0.24		0.76		1.20		0.88		1.41	
CXLVIII,	0.36		0.62		1.40		3.26		6.06	
CXLIX,	0.26		0.98		1.50		2.32		2.44	
CL,	0.16		0.71		1.03		2.17		2.33	
CLI,	0.08		0.14		0.44		0.58		0.78	
CLII,	0.08		0.23		0.17		0.52		0.53	
CLIII,		0.06	0.16		0.36		0.65		1.23	
CLIV,	0.11		0.14		0.30		0.46		0.65	
CLV,	0.00		0.19		0.29		0.46		1.91	
CLVI,	0.00		0.18		0.25		0.55		0.83	
CLVIII,	0.05		0.10		0.48		0.34		0.78	
CLIX,		0.05	0.30		0.54		0.96		0.98	
CLXXVII,	0.05		0.16		0.39		0.42		0.71	
CLXXIX,		0.04	0.31		0.55		0.92		1.32	
5,	0.19		0.85		1.14		0.95		1.63	
XVI,	0.22		0.59		1.01		0.73		1.15	
XXXIII,		0.06	0.33		0.45		0.67		1.17	
XXXVI,	0.18		0.87		0.79		1.10		1.71	
XXXVIII,			0.20		0.30		1.35		1.25	
L,	0.04		0.14		0.36		0.42		0.93	
LI,		0.04	0.32		0.88		0.78		0.94	
LIII,	0.17		0.11		0.29		0.86		1.43	
LIV,		0.05	0.31		1.05		1.24		1.21	
LVI,	0.23		0.41		0.55		1.17		1.61	

TABLE 52. ACID AND ALKALI PRODUCED IN 2% LACTOSE BOUILLON
Fermentation tubes

Strain number	1 day		2 days		4 days		10 days		20 days	
	Acid	Alk.	Acid	Alk.	Acid	Alk.	Acid	Alk.	Acid	Alk.
CXII,		0.30		0.66		0.38		0.28		0.18
CXV,		0.08		0.04	0.06		0.70		0.13	
CXXVII,		0.42		0.42		0.28	0.04		0.12	
CXXVIII,		0.40		0.34		0.33	0.45		0.49	
CXXIX,		0.34		0.49		0.23		0.04		0.17
CXXX,		0.22		0.24		0.18	0.01		0.37	
CXXXIII,		0.40		0.32		0.24		0.16		0.05
CXXXIV,		0.40		0.40		0.60		0.62		0.31
CXXXV,										0.23
CXXXVII,										0.31
CXXXVIII,										0.42
CXXXIX,										0.30
CXL,										
CXLI,									1.57	
CXLII,										0.52
CXLIII,										0.23
CXLV,									0.18	
CXLVI,										0.32
CXLVII,									0.56	
CXLVIII,									0.33	
CXLIX,										0.54
CL,										0.46
CLI,										0.36
CLII,										0.42
CLIII,										0.36
CLIV,										0.34
CLV,										0.20
CLVI,										0.36
CLVIII,										0.50
CLIX,										0.45
CLXXVII,										0.29
CLXXIX,										0.14
5,									0.32	
XVI,									0.11	
XXXIII,										0.45
XXXVI,										0.25
XXXVIII,									0.19	
L,										0.38
LI,										0.44
LIII,										0.34
LIV,										0.62
LVI,										0.75

TABLE 53. ACID AND ALKALI PRODUCED IN 2% LACTOSE BOUILLON
Test tubes, 12 cc.

Strain	1 day		2 days		4 days		10 days		20 days	
	Acid	Alk.	Acid	Alk.	Acid	Alk.	Acid	Alk.	Acid	Alk
CXII,	0.12		0.27		0.35		0.12			
CXV,	0.12		0.11	0.15			0.28			
CXXVII,	0.25		0.30		0.11			0.18		
CXXVIII,	0.18		0.11		0.01		0.03			
CXXIX,	0.21		0.03	0.17			0.35			
CXXX,	0.17		0.07	0.19			0.32			
CXXXIII,	0.20		0.07	0.21						
CXXIV,	0.26		0.31	0.03						
CXXXV,	0.29		0.28		0.21					
CXXXVII,	0.26		0.32		0.35					
CXXXVIII,	0.34		0.35	0.08				0.22		
CXXXIX,	0.22		0.19		0.16		0.20			
CXL,	0.12		0.12		0.36			0.50		
CXLI,	0.14		0.23		0.40			0.43		
CXLII,	0.22		0.31		0.39			0.43		
CXLIII,	0.13		0.19		0.28			0.54		
CXLV,	0.18		0.20	0.11			0.31			
CXLVI,	0.11		0.13		0.35			0.32		
CXLVII,	0.24		0.26		0.34			0.53		
CXLVIII,	0.21		0.11	0.11			0.29			

TABLE 51. ACID AND ALKALI PRODUCED IN 2% SUCROSE BOUILLON
Test tubes, 10 cc.

	1 day		2 days		4 days		10 days		20 days	
Strain	Acid	Alk.	Acid	Alk.	Acid	Alk.	Acid	Alk.	Acid	Alk.
CXII,		0.12		0.40		0.18		0.24		0.31
CXV,		0.16		0.00	0.34		0.80		0.79	
CXXVII,		0.13		0.14			0.14			0.11
CXXVIII,		0.04		0.21				0.03		0.06
CXXIX,		0.01	0.05		0.37		0.82		0.09	
CXXX,		0.00			0.38			0.09	0.10	
CXXXIII,		0.16			0.37		0.52		0.06	
CXXXIV,		0.13		0.22	0.07		0.35		0.21	
CXXXV,		0.22		0.19	0.19		0.33		0.19	
CXXXVII,		0.19		0.18	0.24		0.52		0.04	
CXXXVIII,		0.28		0.30		0.11		0.10		0.11
CXXXIX,		0.26		0.22			0.27		0.24	
CXL,		0.10		0.18		0.32		0.56		0.41
CXLI,		0.11		0.27		0.32		0.12		0.59
CXLII,		0.16		0.34		0.36		0.66		0.43
CXLIII,		0.16		0.18		0.29		0.59		0.59
CXLV,		0.23		0.26	0.11		0.30			0.00
CXLVI,	0.04			0.21		0.48		0.52		0.72
CXLVII,		0.21		0.17		0.36		0.56		0.67
CXLVIII,		0.07	0.05		0.24		0.78			0.12
CXLIX,		0.11		0.05	0.12		0.14		0.14	
CL,		0.13		0.07			0.07			
CLI,		0.07		0.16		0.16		0.12		0.18
CLII,		0.17		0.16		0.17		0.03	0.08	
CLIII,		0.09	0.09		0.15		0.24		0.18	
CLIV,		0.08	0.01			0.03	0.44			
CLV,		0.04		0.01	0.14		0.21		0.15	
CLVI,		0.09		0.07		0.02	0.19			0.37
CLVIII,		0.13		0.14		0.31	0.07		0.06	
CLIX,		0.01	0.09		0.12		0.60			
CLXXVII,		0.17		0.21		0.28	0.58		0.70	
CLXXIX,		0.07	0.08		0.23			0.58		0.19
5,		0.11		0.06		0.10	0.05		0.14	
XVI,		0.07		0.26		0.41		0.69		0.68
XXXIII,		0.10		0.16		0.26		0.31		0.37
XXXVI,		0.12		0.02		0.06	0.97		1.20	
XXXVIII,	0.03		0.11		0.29		1.24		1.47	
L,		0.08		0.27		0.36		0.77		0.68
LI,	0.00	0.00		0.20		0.40		0.71		0.63
LIII,		0.07	0.07		0.40			0.79	0.13	
LIV,		0.02		0.21		0.31		0.72		0.72
LVI,		0.02		0.02		0.00		0.27		0.35

TABLE 55. ACID AND ALKALI PRODUCED IN 5% GLYCERIN BOUILLON
Test tubes, 10 cc.

	1 day		2 days		4 days		10 days		19 days	
Strain	Acid	Alk.	Acid	Alk.	Acid	Alk.	Acid	Alk.	Acid	Alk.
CXII,			0.00		0.25		1.35		1.56	
CXV,			0.00		0.29		0.56		0.53	
CXXVII,				0.06	0.00		0.62		0.94	
CXXVIII,				0.13	0.04		0.47		0.26	
CXXIX,			0.05		0.38		1.26		1.68	
CXXX,			0.00		0.29		0.68		0.56	
CXXXIII,			0.00		0.31		1.16		1.57	
CXXXIV,				0.02	0.14		0.73		0.65	
CXXXV,				0.15	0.07		0.39		1.10	
CXXXVII,				0.12	0.18		0.90		1.36	
CXXXVIII,				0.14		0.03	0.28		0.49	
CXXXIX,				0.00	0.20		1.16		1.06	
CXL,				0.21		0.45		0.37		0.42
CXLI,				0.26		0.39		0.29		0.14
CXLII,				0.24		0.37		0.46		0.55
CXLIII,				0.24		0.33		0.12		0.28
CXLV,				0.08	0.07		0.83		1.12	
CXLVI,				0.27		0.44		0.52		0.23
CXLVII,				0.19		0.22	0.29		0.22	
CXLVIII,			0.13		0.27		1.07		1.39	
CXLIX,			0.00		0.26		1.39		2.08	
CL,			0.03		0.54		1.43		1.05	
CLI,				0.18		0.07	0.43		0.77	
CLII,				0.13		0.04	0.19		0.33	
CLIII,				0.02	0.34		0.88		1.31	
CLIV,				0.00	0.16		0.70		0.96	
CLV,				0.12		0.05	0.19		0.37	
CLVI,			0.06		0.02		0.90		1.13	
CLVIII,				0.12	0.11		0.49		0.67	
CLIX,			0.18		0.26		0.98		1.69	
CLXXVII,				0.19		0.20	0.19		0.34	
CLXXIX,			0.14		0.60		1.00		1.35	
5,						0.13	0.21		0.44	
XVI,						0.27		0.17	0.00	
XXXIII,						0.14	0.13			
XXXVI,						0.15	0.08		0.26	
XXXVIII,						0.20	0.51		0.52	
L,						0.29		0.32		0.16
LI,						0.16	0.23		0.32	
LIII,					0.28		0.95		1.06	
LIV,						0.28		0.09	0.05	
LVI,					0.27		1.38		1.81	

TABLE 56. ACID AND ALKALI PRODUCED IN 5% GLYCERIN BOUILLON
Duplicate trials Test tubes, 10 cc.

	1 day		2 days		4 days		10 days		20 days	
Strain	Acid	Alk.	Acid	Alk.	Acid	Alk.	Acid	Alk.	Acid	Alk.
CXII,					0.30		1.08			
CXV,					0.25		0.54			
CXXVII,					0.04		0.03			
CXXVIII,					0.06		0.34			
CXXIX,					0.34		0.86			
CXXX,					0.35		0.51			
CXXXIII,					0.25		0.67			
CXXXIV,					0.32		0.86			
CXXXV,						0.16	0.17			
CXXXVII,					0.14		0.44			
CXXXVIII,						0.05		0.02		
CXXXIX,					0.05		0.32			
CXL,						0.37		0.62		
CXLI,						0.45		0.62		
CXLII,						0.58		0.62		
CXLIII,						0.37		0.62		
CXLV,						0.08	0.14			
CXLVI,						0.45		0.57		
CXLVII,						0.49		0.43		
CXLVIII,						0.00	0.48			
CXLIX,					0.05		0.86			
CL,					0.25		0.58			
CLI,						0.20	0.37			
CLII,						0.20	0.11			
CLIII,					0.05		0.33			
CLIV,					0.26		0.26			
CLV,						0.08		0.02		
CLVI,						0.15	0.44			
CLVIII,						0.09	0.13			
CLIX,					0.18		0.47			
CLXXVII,						0.19		0.16		
CLXXIX,					0.30		0.89			
5,						0.30		0.20		
XVI,						0.43		0.56		
XXXIII,						0.18	0.20			
XXXVI,						0.19	0.08			
XXXVIII,						0.18	0.17			
L,						0.47		0.45		
LI,						0.15		0.15		
LIII,					0.14		0.59			
LIV,						0.35		0.46		
LVI,					0.60		1.23			

2. *Ammonia production.*—Ammonia was produced by all strains on nutrient broth, which is in accord with the statements of Thumm (31) (compare 20:754). The test was carried out as follows: For the first of the series 125 cc. portions of nutrient broth in Kjeldahl flasks were inoculated and incubated 10 days in the dark at room temperature (22° to 25° C.). At the end of this time determinations were made. Table 57 shows the ammonia content of cultures and control in cc. of N/10 hydrochloric acid equivalent for the 125 cc. and it is also calculated to a 100 cc. basis.

TABLE 57. AMMONIA PRODUCTION

Organism number	cc. N/10 HCl equivalent for 125 cc.	cc. N/10 HCl equivalent for 100 cc.
CXIV,	33.0	26.4
CXV,	23.3	18.6
CXXVII,	24.5	19.6
CXXVIII,	28.9	23.1
CXXIX,	45.6	36.5
CXXX,	31.2	25.0
CXXXIII,	54.7	43.8
CXXXIV,	57.8	46.2
CXXXV,	34.2	27.4
CXXXVII,	41.4	33.1
CXXXVIII,	34.8	27.8
CXXXIX,	43.2	34.6
CXL,	7.6	6.1
CXLI,	9.8	7.8
CXLII,	10.6	8.5
Control,	3.0	2.4

For the remainder of the series cultures were treated as before except that 100 cc. of media were used and the incubation period was 12 days at 20° C. The medium was identical with that of the preceding series.

TABLE 58. AMMONIA PRODUCTION

Organism number	cc. N/10 HCl equivalent for 100 cc.	Organism number	cc. N/10 HCl equivalent for 100 cc.
CXLIII,	13.0	CLXXVII,	23.5
CXLV,	43.8	CLXXIX,	46.3
CXLVI,	9.5	5,	33.3
CXLVII,	13.8	XVI,	11.6
CXLVIII,	39.4	XXXIII,	17.2
CXLIX,	22.5	XXXVI,	14.8
CL,	32.6	XXXVIII,	10.7
CLI,	22.2	L,	7.8
CLII,	14.2	LI,	7.7
CLIII,	48.1	LIII,	49.0
CLIV,	44.6	LIV,	9.8
CLV,	26.0	LVI,	63.5
CLVI,	39.1	CXII,	34.9
CLVIII,	44.7	CXV,	10.6
CLIX,	49.7		
Control,	2.5	Control,	3.4

3. *Action on nitrates.*—Nitrates in nitrate broth were reduced to nitrites by three strains, as indicated by the iodo starch reaction (27:63). All cultures showed ammonia with Nessler's reagent; but this was thought to have been an end product of proteid decomposition rather than of nitrate reduction. No gas was produced.

Two formulas for nitrate broth were used with similar results.

I.	Distilled water	1000. cc.
	Liebig's extract of meat	3. gr.
	Witte's peptone	10. gr.
	Potassium nitrate	3. gr.
II.	Distilled water	1000. cc.
	Witte's peptone	1. gr.
	Potassium nitrate	2. gr.

Cultures in media of the first type were tested at the end of 10 days and 30 days. Only strain LI responded with a positive reaction at the first test, but strains LI, LIV, and XXXVI showed prompt blackening when tested after 30 days. The reaction was more rapid in media prepared by formula II and a positive reaction was obtained in 5 day old cultures of strains LI, LIV and XXXVI. Only these three strains showed the presence of nitrites at any time on either medium. The test

were made repeatedly after incubation periods of various duration. The diphenylamine test for nitrates (32:340) gave a positive reaction on cultures of all strains except XXXVI. (Compare 19:152-154).

4. *Indol.*—Indol was not detected in broth cultures of any strain on the second or tenth day. The 10 cc. cultures were tested with ten drops of chemically pure sulphuric acid and one cc. of .02% sodium nitrite solution. No reaction appeared at the end of ten minutes or after warming. (3:33).

5. *Temperature relations.*—The optimum temperature for all strains was found to be in the neighborhood of 25° C. The thermal death point was approximately determined in the preliminary studies to lie between 40° and 55° C. The minimum temperature for growth was below 3° C. except for strain CXV which did not develop below 5° C. This strain showed growth at 37° C. All others failed to develop at that temperature and eighteen days exposure was fatal to them. Luxwolda (18) states that *B. fluorescens liquefaciens* develops remarkably fast at low temperature (3° to 5° C.).

6. *Acids and alkalies.*—A small amount of acid was produced in dextrose nutrient broth and indications of acid were also observed in nutrient broth containing lactose, sucrose, and glycerin. Titrations were made in hot solution with N/20 sodium hydroxid against phenolphthalein after 1, 2, 4, 10, and 20 days incubation. A trace of acid from proteid decomposition was observed with some of the strains in the sugar free bouillon series.

Hydrogen sulphid.—Tubes of nutrient broth were prepared in the usual way and, after inoculation, a strip of filter paper moistened with lead acetate solution was suspended above the culture in each tube. The paper in cultures of strain XVI began to blacken at five days showing hydrogen sulphid production. In ten days the papers in cultures of strains CXL, CXLI, CXLII, CXLIII, CXLVI, XVI, LIV, CXV, showed positive reactions, which in the cases of the last two strains,

PLATE XIII.—*Ps. fluorescens.* Types of gelatin colonies: liquefaction well under way. Figure 1. Strain LIII (three day old colony). Figure 2. Strain XXXVI (four day old colony). (See pages 567-568.) × 75.

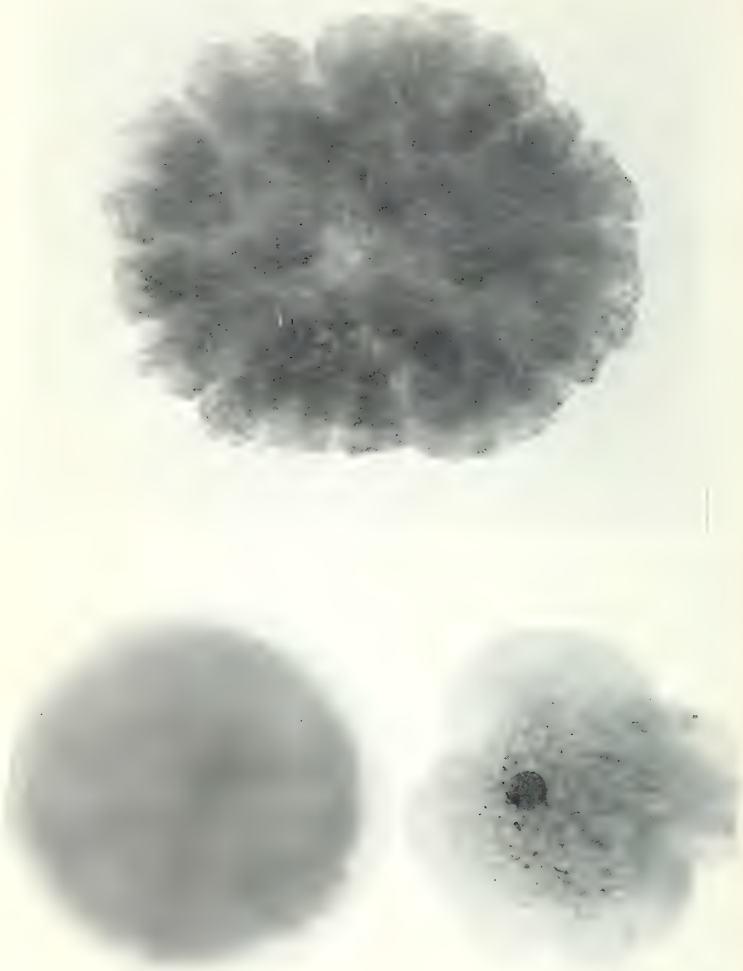

PLATE XIV.—*Ps. fluorescens.* Types of gelatin colonies. Figure 1. Strain XXX (two day old colonies). Figure 2. Strain L (two day old colonies). Figure 3. Strain LIII (two day old colonies). (See pages 567-568.) × 75.

however, amounted to only a trace. After 19 days the papers in CXV, CXL, CXLI, CXLII, CXLIII, CXLVI, XVI, and L showed considerable blackening while those in LI, LIV and XXXVI showed but a trace. Test papers in the controls remained unchanged.

Ammonia was produced by all strains, see pp. 542, 543.

7. *Reduction processes.*—Litmus in litmus milk and in litmus agars was somewhat reduced by all strains. Nitrates were reduced to nitrites by three strains. (See action on nitrates, page 543).

8. *Odor.*—A putrefactive odor was associated with development on all the common media.

9. *Gas production.* None observed by the Smith fermentation tube method in dextrose, lactose, sucrose, glycerin, or nitrate broths.

10. *Crystals.*—These were common in agar stab and plate cultures, and in gelatin plate cultures. Twinned crystals of magnesium ammonium phosphate were formed in Cohn's solution by strains LI and LIV.

11. *Oxygen requirements.*—Agar stab cultures were exposed to an atmosphere freed of oxygen by Buchner's pyrogallic acid method (33). The tubes were placed in liter Novy jars containing 10 grams of dry pyrogallic acid, to which 150 cc. of 1% sodium hydroxid solution was then added and the jars immediately sealed. These were opened at the end of two weeks incubation at room temperature. A barely visible surface colony and a slight growth along the puncture was apparent with all strains. The growth was limited and without fluorescence. While this property was apparent 24 hours later, its failure to develop in the oxygen free atmosphere might be attributed to the limited metabolism rather than to the absence of oxygen. Repeated trials yielded confirmatory results, but other methods (pages 588 and 589) have shown the organisms to be strictly aerobic. The slight growth obtained by the pyrogallic acid method is attributed to the presence of small amount-

of unabsorbed oxygen during the first hours of the experiments.

12. *Fluorescence.*—The fluorescing property was possessed by the strains in varying degrees. Some were never observed to fluoresce with the intensity which was typical of others. The degree of fluorescence was also variable with the same strain upon different media. Strain CXV was never more than doubtfully fluorescent, but it was included in the studies for comparative purposes with the idea that its ability to fluoresce might be intensified by proper cultural methods. This result has not been obtained, however, and for this and other reasons it is probable that the organism in question should not be included with the fluorescent group.

The strongly fluorescent strains in general were the rapid liquefiers and digesters, and they produced more ammonia than the weakly fluorescent ones (c. f. chart page 551). Addition of ammonia or sodium hydroxid generally brought out a bright yellow-green fluorescence in all vigorous cultures deficient in this respect, with the exception of those of strain CXV (31). The pigment disappeared in all cultures if made slightly acid, only to reappear as soon as a slight excess of alkali was present. In general upon agar and broth a beautiful yellow-green fluorescence appeared in from three to five days. On gelatin, fluorescence developed only to a slight degree.

The green fluorescent pigment.—The most extended investigations concerning the pigment formed by the fluorescent bacteria have been made by Gessard (9), Thumm (31), and Jordan (14 and 15). Gessard concluded that *B. pyocyaneus* produces two pigments, pyocyanin and fluorescine. In order to bring out clearly the nature of the fluorescent pigment the papers of Thumm and Jordan (14) are quite extensively cited.

Thumm studied the following species:

B. fluorescens tenuis.
B. fluorescens putidus.
B. fluorescens albus.
B. fluorescens erythrosporus.

B. pyocyaneus (several strains)
Bact. syncyaneum.
B. viridans.

His results may be summarized as follows: All fluorescent bacteria show in alkaline gelatin, first a sky blue, later a moss green fluorescence and, accompanying the latter, a yellowing of the substratum. Old cultures with the exception of those of *B. fluorescens putridus* are orange-red with dark green fluorescence. All these colors are due to one yellow pigment. When an acid producer and a fluorescent organism are cultivated together the yellow pigment is formed normally, but there is no fluorescence. The green fluorescence in every case is caused by the action of the ammonia produced. Calcium chlorid is unessential for the formation of the pigment, but magnesium sulphate and potassium phosphate are of the greatest importance. *Bact. syncyaneum* forms two pigments, a fluorescent one, and a steel-blue one.

Jordan (14) worked with the following species:

B. fluorescens albus.
B. fluorescens tenuis.
B. fluorescens mesentericus.
B. fluorescens putridus.
B. fluorescens liquefaciens.
B. viridans.

He summarizes his results as follows:

"Both sulphur and phosphorus are essential to pigment formation but the nature of the base associated with these elements is not important. Other things being equal the presence of the methyl or methylene group is coincident with superior nutritive value and fluorescigenic power. Acid in the medium not merely conceals the existence of the substance to which the color is due but interferes with those vital activities of the bacilli which in an alkaline medium lead to the production of that substance. An excess of substance favorable to growth and pigment formation checks the latter though the former may be

greater than before." Jordan believes that the fluorescent property is purely incidental. "When the medium contains certain compounds the metabolic activities of the organisms adjust themselves to these conditions and it is of no physiological significance that one of the products of this activity happens to be fluorescent."

13. *Diastasic action on potato starch.* A thin starch paste containing 2% thymol was added to equal volumes of ten day old broth cultures, the tubes placed in the thermostat for 8 hours, filtered and the filtrate tested with Fehling's solution. Sugar was detected in none of the cultures, indicating that diastase had not been formed.

14. *Group numbers.*—The characteristics of the several strains detailed above correspond as follows.

12	strains	to	the	number,	*Pseudomonas*	221.2332132
12	"	"	"	"	"	221.2332133
7	"	"	"	"	"	221.2322132
4	"	"	"	"	"	221.2322133
2	"	"	"	"	"	221.2333133
1	"	"	"	"	"	221.2323132
2	"	"	"	"	"	221.2222132
1	"	"	"	"	"	221.2232133
1	"	"	"	"	*Bacillus*	221.2222232

However since there is no sharp line between acid production and no acid production from the sugars and glycerin, the only specific difference brought out by the group number is in regard to nitrate reduction. If gelatin cultures had been held only six weeks to determine liquefaction, the group number of strains CXL, CXLI, CXLII, CXLIII, CXLVI, XVI, and L, would have been 222.2332133 and of strains LI and LIV 222.2333133. For the group number corresponding to the respective strains, see page 550.

15. *Separation of the 42 strains into groups.*—The 42 strains naturally separate themselves into 6 groups with respect to the following properties: the production of hydrogen sulphid, the reduction of nitrates to nitrites, growth upon asparagin Uschinsky solution, Cohn's nutrient solution, gelatin,

and milk. The separation of the strains into groups is shown in the chart on the following page, where the relation existing between ammonia production and fluorescence is also brought out. In general the strains producing comparatively small amounts of ammonia are the tardy liquefying and feebly fluorescent ones.

CHART SHOWING THE SEPARATION

Strain	Group number	Hydrogen sulphid	Nitrogen from asparagin in Uschinsky solution	Nitrate reduction
CXXVII,	221.2332133	—	—	—
CXXVIII,	221.2332133	—	—	—
CXXX,	221.2332132	—	—	—
CXXXIV,	221.2332132	—	—	—
CXXXV,	221.2322133	—	—	—
CXXXVII,	221.2322132	—	—	—
CXXXVIII,	221.2332133	—	—	—
CXXXIX,	221.2322132	—	—	—
CXLV,	221.2222132	—	—	—
CL,	221.2332132	—	—	—
CLI,	221.2332132	—	—	—
CLIII,	221.2322132	—	—	—
CLIV,	221.2322132	—	—	—
CLV,	221.2332133	—	—	—
CLVI,	221.2322132	—	—	—
CLVIII,	221.2332133	—	—	—
CLXXIX,	221.2332132	—	—	—
XXXIII,	221.2332132	—	—	—
XXXVIII,	221.2322132	—	—	—
LIII,	221.2332132	—	—	—
CXII,	221.2332132	—	—	—
CXLVII,	221.2232133	—	—	—
CLII,	221.2332132	—	—	—
CXXIX,	221.2322133	—	+	—
CXXXIII,	221.2332132	—	+	—
CXLVIII,	221.2222132	—	+	—
CXLIX,	221.2332132	—	+	—
CLIX,	221.2322132	—	+	—
CLXXVII,	221.2322133	—	+	—
5,	221.2322133	—	+	—
LVI,	221.2332132	—	+	—
XXXVI,	221.2323132	±	±	+
LI,	221.2333133	+	±	+
LIV,	221.2333133	±	—	+
CXL,	221.2332133	+	±	±
CXLI,	221.2332133	+	—	—
CXLII,	221.2332133	+	—	—
CXLIII,	221.2332133	+	—	—
CXLVI,	221.2332133	+	+	—
XVI,	221.2332133	+	—	—
L,	221.2332133	+	—	—
CXV,	221.2222?32	+	—	—

+, present; —, absent; ±, trace.

OF THE 42 STRAINS INTO GROUPS

Gelatin liquefaction begins in	Digestion of milk begins in	Ammonia in 100 cc. broth, 10-12 days, cc. N/10 HCl equivalent	Growth on Cohn's nutrient solution	Fluorescence
24 hrs.	10 d.	19.6	—	strong
16 hrs.	6 d.	23.1	—	"
16 hrs.	6 d.	25.0	—	"
16 hrs.	10 d.	46.2	—	"
24 hrs.	10 d.	27.4	—	"
24 hrs.	6 d.	33.1	—	"
62 hrs.	10 d.	27.8	—	"
24 hrs.	10 d.	34.6	—	"
24 hrs.	10 d.	43.8	—	"
16 hrs.	6 d.	32.6	—	"
40 hrs.	10 d.	22.2	—	"
16 hrs.	10 d.	48.1	—	"
16 hrs.	10 d.	44.6	—	"
20 hrs.	10 d.	26.0	—	"
16 hrs.	10 d.	39.1	—	"
16 hrs.	7 d.	44.7	—	"
20 hrs.	6 d.	46.3	—	"
40 hrs.	10 d.	17.2	—	moderate
16 hrs.	8 d.	10.7	—	feeble
16 hrs.	10 d.	49.0	—	strong
10 d.	10 d.	26.4	—	"
30 d.	30 d.	13.8	—	feeble
10 d.	10 d.	14.2	—	moderate
16 hrs.	10 d.	36.5	—	strong
16 hrs.	10 d.	43.8	—	"
16 hrs.	10 d.	39.4	—	"
16 hrs.	10 d.	22.5	—	"
20 hrs.	8 d.	49.7	—	"
24 hrs.	8 d.	23.5	—	"
20 hrs.	10 d.	33.3	—	"
16 hrs.	5 d.	63.5	—	"
24 hrs.	30 d.	14.8	—	feeble
50 d.	30 d.	7.7	+	"
50 d.	30 d.	9.8	+	"
5-6 mo.	3 mo.	7.6	—	feeble
5-6 mo.	3 mo.	9.8	—	"
5 mo.	3 mo.	10.6	—	"
4-5 mo.	3 mo.	13.0	—	"
5-6 mo.	3 mo.	9.5	—	"
4-5 mo.	40 d.	11.6	—	"
5 mo.	3 mo.	7.8	—	"
16 hrs.	10 d.	23.3	—	doubtful

COMPARATIVE STUDIES OF SEVEN REPRESENTATIVE STRAINS OF GREEN FLUORESCENT SAP BACTERIA AND SIX KNOWN SPECIES.

After having separated the 42 strains into groups in the preliminary studies as shown above, 7 representative strains were chosen for a more thorough study of their morphological, cultural, physical and biochemical features. The strains selected follow: From group one, strain CXLV, capsulated, and strain CXII, a slow liquefier, non-capsulated; from group two, strain CXLVIII a rapid liquefier, non-capsulated and, in contrast to the other groups, able to grow in asparagin Uschinsky solution; from group three, strain XXXVI, a nitrate reducer which does not grow on Cohn's solution; from group four, strain LI, a nitrate reducer which grows on Cohn's solution; from group five, strain CXL, a very tardy liquefier and casein digester, producing abundant hydrogen sulphid, but not reducing nitrates; from group six, strain CXV, a strong hydrogen sulphid producer, a rapid liquefier, growing at 37° C., Gram positive, and at most only doubtfully fluorescent. In addition, 6 strains of known species were introduced for comparative purposes. These are as follows:

Ps. alba	(Zimmermann)	Migula.
Ps. fluorescens	(Flügge)	"
Ps. longa	(Zimmermann)	"
Ps. mesenterica	(Tataroff)	"
Ps. tenuis	(Zimmermann)	"
Ps. putrida	(Flügge)	"

The first five cultures were obtained from Kral's Laboratorium, while the culture of *Ps. putrida*, added to the station series some years ago, was supplied by Novy.

The 13 strains were rejuvenated by preliminary cultivation, while frequent replating assured the purity of the cultures. For methods and procedures see pages 525 and 526. The Society Card was used as a basis for the comparative studies and, in general, as in the preliminary studies, the methods em-

ployed were those recommended in "Standard Methods of Water Analysis."

DETAILED FEATURES

I. MORPHOLOGY

1. *Vegetative cells.*—Observations were made on 24 hour agar hanging block cultures and on hanging drop preparations from 16 to 24 hour agar slant condensation water. The cells of all strains were motile rods of medium length with rounded ends, occurring typically in chains of two; less frequently singly, or in chains of four or eight. Measurements were made of what appeared to be individual cells, but in some cases the figures given may represent double organisms. The limits of size which were obtained are best shown in tabular form:

TABLE 59. LIMITS OF SIZE ON HANGING BLOCK

Strain	Maximum		Minimum	
	Length	Diameter	Length	Diameter
Ps. alba,	5.0 microns	1.2 microns	1.7 microns	0.7 microns
Ps. fluorescens,	3.3 "	0.9 "	2.3 "	0.6 "
CXII,	4.7 "	1.0 "	2.0 "	0.6 "
CXV,	2.6 "	1.0 "	1.0 "	0.8 "
CXL,	2.3 "	0.9 "	1.0 "	0.5 "
CXLV,	4.0 "	0.7 "	1.5 "	0.6 "
CXLVIII,	4.0 "	0.6 "	1.4 "	0.4 "
XXXVI,	2.3 "	0.7 "	1.6 "	0.6 "
LI,	3.3 "	0.7 "	1.7 "	0.5 "

TABLE 60. LIMITS OF SIZE ON HANGING DROPS

Strain	Maximum		Minimum	
	Length	Diameter	Length	Diameter
Ps. alba,	2.5 microns	0.8 microns	1.25 microns	0.5 microns
Ps. fluorescens,	3.0 "	0.8 "	1.18 "	0.6 "
Ps. longa,	2.2 "	0.7 "	1.0 "	0.6 "
Ps. mesenterica,	1.6 "	0.8 "	1.05 "	0.6 "
Ps. tenuis,	1.9 "	0.8 "	1.05 "	0.5 "
Ps. putrida,	1.1 "	0.7 "	0.9 "	0.5 "
CXII,	1.5 "	0.6 "	0.9 "	0.5 "
CXV,	4.0 "	0.9 "	1.6 "	0.5 "
CXL,	2.3 "	0.9 "	1.0 "	0.5 "
CXLV,	3.0 "	0.8 "	0.9 "	0.5 "
CXLVIII,	3.8 "	0.8 "	1.5 "	0.5 "
XXXVI,	1.5 "	0.7 "	0.7 "	0.5 "
LI,	3.0 "	0.8 "	1.2 "	0.6 "

Measurements upon preparations from 24 hour agar slant-stained two minutes with carbol fuchsin without heating yielded the following results.

TABLE 61. LIMITS OF SIZE ON STAINED PREPARATIONS

Strain	Maximum Length	Maximum Diameter	Minimum Length	Minimum Diameter
Ps. alba.	2.8 microns	0.7 microns	1.1 microns	0.4 microns
Ps. fluorescens,	2.1 "	0.5 "	1.1 "	0.4 "
Ps. longa,	1.9 "	0.7 "	1.3 "	0.5 "
Ps. mesenterica.	1.7 "	0.7 "	0.9 "	0.5 "
Ps. tenuis.	2.5 "	0.8 "	1.7 "	0.5 "
Ps. putrida.	1.4 "	0.7 "	0.9 "	0.5 "
CXII,	2.2 "	0.6 "	1.2 "	0.5 "
CXV,	2.0 "	0.8 "	0.8 "	0.5 "
CXL,	1.4 "	1.0 "	0.7 "	0.5 "
CXLV,	3.2 "	0.7 "	1.0 "	0.5 "
CXLVIII,	2.7 "	0.7 "	1.8 "	0.6 "
XXXVI,	1.8 "	0.6 "	1.1 "	0.5 "
LI,	1.5 "	0.6 "	1.1 "	0.5 "

Flügge (6:292) states that *B. fluorescens liquefaciens* is .3-.5 microns in diameter and 1-2 microns in length. According to Migula (21:886) *Ps. fluorescens* has the following dimensions: diameter .68 microns; length 1.17-1.86 microns; Chester (3:323) gives the following: 1-1.5 microns in length by .5 microns in diameter. Ellis (5:108) gives the length of the rods from 1.5-6 microns and the diameter .4 microns. Zimmermann (34:16) gives the following measurements for *B. fluorescens tenuis.* Length 1-1.85 microns; thickness approximately .8 microns.

2. *Endospores.*—No indication of endospores was observed in any strain. The various spore stains which were tried failed to show anything further than polar bodies or granulation of rods. Zimmermann notes the presence of polar granules in *B. fluorescens tenuis* (34:16).

3. *Flagella.*—Flagella preparations were made only upon the sap bacteria. From 1 to 6 polar flagella were easily demonstrated upon each strain except CXV. (See Plate X, figures 5 and 6). Preparations were made from 16 to 24 hour agar

slants by means of Loeffler's method (with anilin gentian violet) or by Löwit's modification of the same. The arrangement of the flagella of the several strains was as follows.

Strain CXII.—A polar tuft of 3 flagella at one end of a chain of two cells was typical. Occasionally tufts of 1 to 6 flagella occurred at both ends of double cells.

Strain CXV.—This strain had peritrichiate flagella. Tufts were common at each end of chains. Lateral flagella were common on single cells (c. f. Plate X, figure 2).

Strain CXL.—The flagella typically occurred in a polar tuft at one end of the double cells, frequently at both ends.

Strain CXLV.—Tufts of flagella were demonstrated on one end of double cells, and occasionally on both ends. Single organisms usually had one polar flagellum.

Strain CXLVIII.—Double cells commonly had 1 or 3 flagella at one end; occasionally 1 at each end. Single cells with 2 polar flagella were not uncommon.

Strain XXXVI.—The double cells had 1, 3, or 6 polar flagella, most commonly a tuft of 3; occasionally the flagella occurred at both ends of the double cells.

Strain LI.—Most commonly 2 flagella occurred at one end of the chains of two cells and 3 at the other, or 1 at one end and 1 to 6 at the other. Double cells with 1 to 6 flagella at one end were common.

4. *Capsules.*—Only the fluorescent sap bacteria were examined for capsules. Such an envelope was demonstrated upon strains CXV and CXLV and upon strain XXXIII as previously noted (page 528). A suggestion of capsulation was noted on flagella preparations of strain CXLVIII, but the results with the contrast capsule stain were negative. Cultures of strain XXXVI on 2% sucrose peptone solution were very stringy.

Strain CXV.—Capsules were easily demonstrated upon preparations from 10 day broth cultures by either Welch's method or by Richard Muir's contrast stain (*22*:106). Fre-

quently 2 and sometimes as many as 5 cells occurred side by side in the same capsule, with flagella arising about the periphery of the envelope (c. f. Plate X, figure 3).

Measurements obtained on the capsule preparations follow:

Length of cell	Diameter of cell	Length of capsule	Diameter of capsule
1.6 microns	0.53 microns	2.18 microns	1.57 microns
1.8 "	0.53 "	2.50 "	1.57 "
1.8 "	0.50 "	2.80 "	1.82 "

Strain CXLV.—Material from 10 day broth cultures was stained by Richard Muir's method and capsulation demonstrated. Parallelism of cells within the capsules was absent with this strain.

5. *Zoogloca.*—Not observed.

6. *Involution forms.*—Unusually long cells were common in old cultures.

7. *Staining reactions.*

Gram's stain.—Films from 4 day agar colonies of the 13 strains were stained as follows: (22:103).

Treated with anilin gentian violet (22:101) 1½ minutes, washed in water; treated with Gram's solution 1½ minutes, and immersed in absolute alcohol 4 minutes. All of the fluorescent organisms were Gram negative (21:888). All strains except *Ps. alba* showed some granular spots which failed to decolorize in 4 minutes. Strain CXV remained deeply stained even after 15 minutes in absolute alcohol.

Gram's stain with amyl alcohol (29:108).—Films of strains CXII, CXV, and CXL were stained as above, but amyl alcohol was used 5 minutes for decolorizing. Strain CXII retained the stain faintly, some cells more than others, and a bi-polar effect was noticeable. With CXV the stain was irregularly retained, the cells having a granular appearance. Cells of strain CXL were only partially decolorized even after 15 minutes exposure to amyl alcohol.

Aqueous fuchsin.—Aqueous fuchsin was prepared by dissolving one gram of Grübler's fuchsin in 10 cc. of water. Films from 4 day agar colonies of the 13 strains were treated with this stain for two minutes and washed in water. The films were mounted in water and microscopic examinations made. The cells of *Ps. fluorescens, Ps. longa, Ps. tenuis,* CXV, CXL, and LI were deeply stained: while those of *Ps. alba, Ps. mesenterica, Ps. putrida,* CXII, CXLV, CXLVIII, and XXXVI were only faintly stained. The cells from all strains except CXV presented a granular plasmolysed appearance and with strains *Ps. tenuis* and CXL a bi-polar staining was common.

Aqueous gentian violet.—The stain was prepared by dissolving 1 gram of Grübler's gentian violet in 10 cc. of water. Preparations from 4 day agar colonies were stained 2 minutes and washed in water. The cells of *Ps. alba, Ps. longa,* CXV, CXL, CXLV, CXLVIII, and LI were well stained, while those of *Ps. fluorescens, Ps. tenuis, Ps. mesenterica, Ps. putrida,* XXXVI, and CXII were rather faintly stained. Plasmolysis occurred with all strains but was particularly noticeable in the following: *Ps. fluorescens, Ps. longa, Ps. tenuis, Ps. putrida,* and CXII.

Carbol fuchsin (Zichl).—Films were prepared from 4 day agar colonies of all strains, stained two minutes with Ziehl's carbol fuchsin and rinsed with water. Strain CXV was the only organism in which the entire cell was stained. Cells of *Ps. alba, Ps. fluorescens, Ps. longa, Ps. putrida* were only faintly stained, while those of *Ps. mesenterica, Ps. tenuis,* CXII, CXL, CXLV, CXLVIII, XXXVI, and LI were fairly well stained but showed a bi-polar effect. Chains of cells of the latter two mentioned strains presented a barred appearance.

II. Cultural Features

1. *Agar stroke.*—On agar stroke the thirteen strains displayed similar growth characters, indeed no specific constant difference can be pointed out. The line of inoculation first ap-

peared in 24 hours, as a faint white streak, rapidly becoming beaded above, moist, glistening and of slimy consistency. It had a white to light grayish color and often appeared nacreous in certain lights. The medium became more or less green fluorescent on the third day, the coloration gradually increasing in intensity. Strain CXV grew more slowly than the others and with doubtful fluorescence.

2. *Potato slants.*—Cylinders were cut from large tubers and divided so as to give a slant surface. These were washed for several hours in flowing tap water before they were placed in the tubes containing a few drops of distilled water. Sterilization was accomplished by the intermittent method.

Upon this medium a moderate growth occurred with all strains. At first in about twenty-four hours a very delicate whitish accumulation could just be distinguished. This developed slowly into a light brown, slimy, smooth, filiform, spreading band, later becoming thick and darker brown, sometimes even chocolate brown in old cultures. Strain CXV was characterized frequently by a more or less corrugated to rhizoid, slimy growth, moist at first, later appearing rather dry. The medium was more or less grayed by all strains. Strong greening of the substratum was present with some strains but not constantly. This reaction was observed with the following strains: CXII, in 31 days: CXLV, CXLVIII, LI in 5 days: *Ps. fluorescens* in 3 days.

3. *Loeffler's blood serum.*—This medium was not employed.

4. *Agar stab.*—Specific differential characters were not found on this medium. The surface growth was moderate with all 13 strains, at first white, becoming more or less brown, round and entire to contoured with toothed edge. The puncture growth which was at first beaded to filiform became echinate to villous, the latter character being typical of old cultures. The best development occurred in the upper portion of the stab. The medium was at first blue-green fluorescent, becoming more

and more yellow-green. In the old cultures the agar had assumed a brownish to amber color. Fluorescence was not as strongly evidenced by some strains as by others. The strongly fluorescent strains were: *Ps. fluorescens*, CXLVIII, CXII, CXLV, *Ps. mesenterica*, CXL, *Ps. tenuis* and LI named approximately in the order of their fluorescing powers. *Ps. alba*, *Ps. longa*, *Ps. putrida*, and XXXVI showed moderate fluorescence. Strain CXV was at most doubtfully fluorescent.

5. *Gelatin stab at 20° C.*—Gelatin media were prepared with "Nelson's Photographic Gelatin No. 1," and a "Gold Label" gelatin. Sometimes 10 and sometimes 12% were employed. Neither the percent of gelatin nor the brand was observed to have any significant effect on the cultural characters. On gelatin media there were two types among the 13 strains, a liquefying and a non-liquefying type, the latter including two very tardy liquefiers. However, a sharp division line between types does not exist.

Liquefying type.—To this group belong *Ps. fluorescens*, *Ps. mesenterica*, CXII, CXV, CXLV, CXLVIII, and XXXVI.

Ps. fluorescens.—In 18 hours a slight growth appeared as a filiform whitish line. Crateriform liquefaction began in two days gradually extending the shallow crater until the liquefaction assumed a stratiform type. The fluid was at first yellowish white and moderately turbid, the stab being filiform and finely villous above, beaded and finely villous below. A slight green fluorescence was apparent, but only in the liquid portions. The fluorescence in gelatin, +10 Fuller's scale, was never as marked as in agar of the same reaction. The influence of the reaction upon fluorescence is apparent from the results of two series of gelatin cultures, one of which had an initial reaction of —4.7 Fuller's scale and the other of —12. The fluorescence appeared earlier and became noticeably more intense in the more alkaline medium, showing that a slightly alkaline gelatin is more favorable to the development of fluorescence than a neutral or acid one. This is in accord with the statements of Thumm (31) and Jor-

dan (14). During the incubation the alkali was partly neutralized by the carbon dioxid absorbed from the air. The actual reaction at the time the notes were taken was determined by titrating control tubes with N/20 sodium hydroxid and phenolphthalein. The medium originally reacting at —4.7 Fuller's scale had an actual reaction of +2.4; that originally reacting at —12.4 had an actual reaction of —6.1. Fluorescence had not appeared in 26 days in the medium having an original reaction of —4.7, but in the medium having an original reaction of—12.4 fluorescence was as marked as in agar cultures. The actual reaction of the more alkaline medium at this time was +2.7 Fuller's scale. Liquefaction has proceeded farther in this medium than in the other, which now had a reaction of +4.4 Fuller's scale.

Gelatin liquefaction proceeded slowly and was completed in 45 days, at which time the fluid was rather clear and of an olive green color. Considerable white flaky precipitate was thrown down as liquefaction proceeded and a scum usually appeared. There was a slight development in the line of puncture, liquefaction occasionally appearing as a tunnel or as beaded spots along the stab.

Ps. mesenterica.—In 1 or 2 days the growth appeared as a white line, filiform above and beaded below. The colony was white with depressed center and irregularly dentate edge, occasionally round with entire edge. Crateriform liquefaction usually began in from 8 to 17 days, but was occasionally delayed until about a month had elapsed. The liquefaction gradually became stratiform and was practically completed in about 4 months. A somewhat more rapid liquefaction and stronger fluorescence occurred in alkaline gelatin; however traces of green fluorescence could be detected occasionally in gelatin reacting +10 Fuller's scale.

Strain CXII.—At the outset of these studies this organism first began gelatin liquefaction in about 10 days; at their close, after an interval of two years, gelatin liquefaction constantly appeared in 24 hours. In one to two days the growth was a

white filiform line which gradually became echinate to villous. Liquefaction proceeded from crateriform to stratiform and was completed in about one month. Fluorescence was rarely seen in +10 gelatin but appeared in alkaline gelatin (compare with *Ps. mesenterica* and *Ps. fluorescens* pages 559, 560). An abundant white flaky precipitate settled out as liquefaction proceeded. Growth in the puncture was transient, the line of inoculation remaining about the same.

Strain CXV.—Growth appeared in a few hours, the puncture being beaded, liquefaction becoming evident in 10 hours. The colony sank in a saucer-like depression, the liquefaction soon becoming stratiform, proceeding quite rapidly at first but gradually slowing down when nearly all the 7 cc. of medium had been liquefied. The line of puncture quite frequently showed considerable liquefaction. The fluid was slightly turbid but developed neither yellow nor green color. A thin surface membrane developed and a moderate amount of white precipitate was thrown down. Liquefaction of the 7 cc. of medium was completed in about a month's time.

Strain CXLV.—This organism at the beginning of the studies was one of the most rapid liquefiers, commencing liquefaction in 24 hours and completing it in 27 days; but in the course of 18 months' cultivation it has gradually lost its ability to liquefy gelatin reacting +10 Fuller's scale. Otherwise it does not differ essentially from strain CXII. In the studies with alkaline gelatin media with original reactions of —4.7 and —12.4 Fuller's scale, this organism showed crateriform liquefaction in 26 days in the —12.4 medium and none in the —4.7 medium. At this time the —12.4 medium had a reaction of +2.7 Fuller's scale. It is thus evident that this organism retained the power to liquefy alkaline or very slightly acid gelatin, after it had practically lost its ability to liquefy the ordinary gelatin. Fluorescence occurred only in the alkaline gelatin.

Strain CXLVIII.—This organism resembled *Ps. fluorescens* very closely on gelatin stab, but in old cultures it did not show

the olive green color noted with the latter organism. No other points of difference were observed.

Strain XXXVI.—This strain could not be distinguished from *Ps. fluorescens* or strain CXLVIII, except by a more feeble fluorescence on alkaline gelatin.

Strain LI.—This organism also resembled *Ps. fluorescens* closely. It could be distinguished only by the fact that liquefaction was delayed, first appearing in about 50 days. LI is evidently a strain of *Ps. fluorescens*, tending toward the variety *non-liquefaciens*.

The non-liquefying type.—To this group belong the following organisms: *Ps. putrida, Ps. alba, Ps. longa.* Strains *Ps. tenuis* and CXL are classified here provisionally since liquefaction is long delayed. Cultures were held under observation for six months in a moist chamber at 18° to 20° C.

Ps. putrida.—Growth was slow, becoming visible the fourth day; the stab was filiform above and beaded below with a small white colony at the surface. At 12 days the colony was round and brownish, the surface being somewhat contoured. At 20 days the colony had become chocolate brown at the center, and the edge showed spiny outgrowths. In from 20 to 40 days the medium showed a strong browning, beginning at the surface and progressing slowly downward. No liquefaction was observed in five months. At this time the colony was round, about 1 cm. in diameter, having a reddish-brown center and raised edge with rhizoid and spiny processes; the puncture was beaded and villous.

Ps. alba and *Ps. longa.*—Cultures were similar to those of *Ps. putrida*, but no browning of the colony or of the medium was apparent. In 5 months the following characters were in evidence; colony, white, round with beaded to lacerate edge; puncture, beaded and widely villous in the upper portions; no liquefaction.

Ps. tenuis.—Young cultures were not essentially different from those of *Ps. alba* and *Ps. putrida*. Some colonies were round and entire, while others were erose. The stab was filiform

to beaded, becoming villous later. Liquefaction constantly began after about 4 months and proceeded slowly, the fluid being thick and slimy. At this time the stab was a faint white filiform line, development in this portion of the culture having ceased. According to Migula's classification *Ps. tenuis* is a non-liquefier. (21:910).

Strain CXL.—Cultures of this organism were like those of *Ps. tenuis.* Liquefaction constantly began after about 5 months' incubation. At this time the stab was either filiform and beaded, or filiform, beaded, and widely villous. As in the cultures of *Ps. tenuis* the liquid was quite thick and slimy. The purity of the culture and its identity with the original strain was proved. It is worthy of note that this organism, when held at 25° C., peptonized milk only after three months' incubation.

6. *Nutrient broth.*—Upon this medium slight differences were observed among the different strains. Moderate clouding appeared in 18 to 24 hours in all except CXV, which was rather tardy in showing growth in most liquid media. The medium rapidly became more or less turbid, forming a scum or pellicle which at intervals settled to the bottom of the tube as a white flaky to viscid precipitate. A rather tough membranous pellicle was produced by stain CXLV. More or less blue-green fluorescence was apparent in from 3 to 10 days, but was never strong in *Ps. longa, Ps. tenuis. Ps. putrida* or XXXVI, except in alkaline broth. Strain CXV produced only a trace of fluorescence. The other strains usually showed beautiful green fluorescence, but it was not constantly present. The blue-green rapidly became yellow-green and in old cultures the medium cleared and showed an amber yellow color. Cultures of CXV and CXLV often became quite stringy.

Strain CXV.—A slight to moderate clouding appeared in from 2 to 5 days, gradually increasing in intensity. Later the cultures developed a white surface membrane, the substratum becoming quite clear. An abundant white precipitate was thrown down. Old cultures became amber colored.

7. *Milk.*—The medium consisted of fresh centrifuged milk, titrated, filtered, and tubed, 10 cc. per tube. Sterilization was effected by the intermittent method.

Acid production.—Triplicate titrations of cultures and controls were made after 1, 2, 4, 10, 20, and 46 days' incubation. Five cc. of cultures and controls respectively were pipetted into 45 cc. of distilled water in Erlenmeyer flasks, 0.5 cc. of 1-100 phenolphthalein added, the mixture boiled 2 minutes and titrated hot with N/20 sodium hydroxid. The averages of the three titrations of cultures were compared with the averages of those of the three control tubes, in order to obtain the percent of normal acid or alkali produced at each time. The results obtained were as follows:

TABLE 62. ACID PRODUCTION IN MILK

Strain	1 day		2 days		4 days		10 days		20 days	
	Acid	Alk.	Acid	Alk.	Acid	Alk.	Acid	Alk.	Acid	Alk.
Ps. alba,		0.02		0.15		0.54		0.89		0.93
Ps. fluorescens,		0.07	0.13		0.46		1.76		3.77	
Ps. longa,		0.22		0.50		0.62		1.10		1.14
Ps. mesent'a.	0.00	0.00		0.30		0.69		0.87		1.11
Ps. tenuis.		0.26		0.31		0.46		0.75		0.83
Ps. putrida,		0.12	0.05			0.03		0.29	0.04	
CXII,		0.26	0.81		1.28		2.80		3.45	
CXV,	0.03		0.05		0.13		0.54		1.20	
CXL,		0.04	0.08			0.10		0.38		0.72
CXLV,		0.29		0.28		0.48		0.44		0.90
CXLVIII,		0.06	0.78		1.46		2.42		3.70	
XXXVI,		0.20		0.28		0.44		0.17	0.74	
LI,		0.03		0.21		0.22		0.56		0.91

Strain	26 days		46 days	
	Acid.	Alk.	Acid	Alk.
Ps. alba		1.15		1.20
Ps. fluorescens,	5.29		4.41	
Ps. longa,		1.03		1.22
Ps. mesenterica,		1.19		1.22
Ps. tenuis,		0.99		0.72
Ps. putrida,		0.06	0.29	
CXII,	3.54		4.29	
CXV,	1.82		3.85	
CXL,		0.62		0.87
CXLV,		0.45		0.12
CXLVIII,	5.31		3.46	
XXXVI,	0.72		2.20	
LI,		0.57		1.22

There were 2 types among the 13 strains according to their action on milk. One type produced considerable acid and coagulated and digested the medium quite rapidly, a more or less greenish color being present. The coagulum was of a more or less jelly-like consistency. The other type showed clearing without coagulation of the milk, the action being delayed and taking place very slowly. With certain strains digestion was not evident until about the third month. To the first group belong the following strains: *Ps. fluorescens*, CXII, CXV, CXLVIII, and XXXVI; and to the second group belong: *Ps. alba*, *Ps. longa*, *Ps. mesenterica*, *Ps. tenuis*, *Ps. putrida*, CXL, CXLV, and LI.

Strains *Ps. fluorescens*, CXII, and CXLVIII of the first type, behaved similarly on milk. A more or less jelly-like coagulation appeared in from 2 to 10 days coincident with acid production. The coagulum formed was rather slimy with the first organism named but with the others was quite firm. Digestion with more or less greenish discoloration of the peptonized portion was apparent in 4 days, becoming complete in from 18 to 30 days, the cultures at this time being relatively clear and closely resembling broth cultures of similar age. A deep green color appeared at the surface in these old cultures and upon agitation the whole medium became olive green. The coloration was more pronounced in *Ps. fluorescens* than in the other two. Luxwolda (18) states that *B. fluorescens liquefaciens* first coagulates milk with a lab ferment and then peptonizes it.

Strain XXXVI differed from the three preceding organisms in that only a trace of green color was present. Coagulation with this strain was always of jelly-like consistency.

Strain CXV showed clearing without coagulation until about the eighteenth day when a firm coagulum appeared coincident with acid production. Digestion continued and was completed in from 30 to 104 days. The medium never appeared green but was more or less straw colored throughout.

With the eight strains of the second type the medium remained practically unchanged in appearance from 10 to 85 days.

the only change which could be noted being its scarcely perceptible browning or greening. Clearing without coagulation occurred sooner or later with all strains, and was complete in from 1 to 5 months' time. Cultures of strain CXL in particular could not be distinguished from the control tubes until about 3 months after inoculation, when clearing without coagulation began to be apparent. This reaction was completed in from 4 to 5 months. Repeated trials with recovery of the organism showed that this clearing of the medium was not due to contamination. (Compare action of this organism on milk with its action on gelatin). The only evidence of growth with strains *Ps. longa*, *Ps. mesenterica*, and *Ps. putrida* until after a month had elapsed, was a scarcely perceptible browning of the medium and a thin scum.

8. *Litmus milk.*—To freshly centrifuged milk with a reaction of +10 to +12 Fuller's scale was added 2% of a saturated aqueous solution of Merck's chemically pure blue litmus; the medium was then filtered, tubed and sterilized by the intermittent method. When ready for use the milk had a rich lavender color.

On this medium all strains developed an alkaline reaction, which, when digestion commenced, was succeeded by one of an acid character. The groups already referred to on gelatin and milk were likewise differentiated here.

The first group, rapid liquefiers and rapid digesters, showed an alkaline reaction at the outset which soon gave place to an acid reaction when digestion commenced. As digestion proceeded, a purple to a reddish coloration advanced into the substratum, the latter becoming first blue, then purple and red, finally bleaching to a straw or amber color when digestion was complete. The surface layers were more or less greenish, especially with *Ps. fluorescens*, CXII, CXLVIII. Upon agitation of the tubes when digestion was completed, the medium became olive green throughout. To this first group belong *Ps. fluorescens*, CXII, CXV, CXLVIII, and XXXVI. *Ps. mesenterica* on this medium reacts more like the second group.

The second group, non-liquefiers, (and very tardy liquefiers and digesters), comprises the following strains: *Ps. alba*, *Ps. longa*, *Ps. putrida*, *Ps. tenuis*, CXL, LI and *Ps. mesenterica*. Alkalinity was apparent the second day, except with *Ps. putrida*; it first manifested this reaction on the tenth day. Alkalinity gradually increased at first in cultures of this group, and after some time acidity developed simultaneously with digestion. After several months, when digestion was nearly complete, cultures of all strains except CXL and *Ps. putrida* were colored purple throughout, without perceptible clearing. Cultures of CXL were pink throughout, but otherwise resembled *Ps. putrida*.

The action of all 13 strains on litmus milk was essentially the same; first, alkali production, then acid production accompanied by digestion.

9. *Gelatin colonies.*—The colony of strain CXLVIII is described as the type of the rapidly liquefying group.

Macroscopically; at first punctiform to round, yellowish, raised, smooth, glistening, and entire, soon liquefying and sinking in saucer-like depressions, the fluid showing slight green fluorescence. Microscopically; round, entire, convex, brownish, center dark and surrounded by a thinner zone (liquid). Internally granular to grumose with spiny processes about the edge. The filaments were variously oriented, being more loosely intertwined at the edge where they extended out into the liquid portion. The colonies soon disintegrated, becoming flaccose or grumose masses in the liquefied gelatin. (See Plate XII XIV).

Ps. fluorescens and strains CXII and CXLV could not be distinguished specifically from CXLVIII.

Strain CXV.—This strain closely resembles the type. Radiate filaments were frequently present, often being almost perfectly symmetrical, the ends of the filaments forming the bordering fringe of the colonies. Liquefaction was very rapid.

Strain XXXVI.—At first round, entire, yellowish brown, finely granular discs, sometimes with an irregular cracked appearance. Some were undulately zoned and the surface irregularly

marked with curved lines. The internal structure became grumose, to broken and fimbriate. A slow and somewhat delayed liquefaction began as a finely granular outer zone, the appearance thereafter conforming to the type, CXLVIII.

Ps. alba is described as the type of the non-liquefying and tardy liquefying group. Macroscopically: punctiform, raised, smooth, yellowish. Microscopically: roundish, entire to broken, yellow-brown, somewhat gray towards the edge. Internally grumose to flocose. *Ps. putrida*: slower growth than *Ps. alba*: macroscopically like the latter. Microscopically: round, entire, brownish, granular. The surface was slightly ridged and furrowed giving a shadowy appearance to the colony. Later the colonies became grumose to broken and were dark brown in color.

Ps. tenuis.—Macroscopically: like *Ps. alba*. Microscopically: roundish, granular to grumose or flocose, becoming spiny or fimbriate.

Strain CXL.—Macroscopically: like *Ps. alba*. Microscopically: much like the type, being round, convex, entire, yellowish-brown, and granular toward the center, the edge being thicker and somewhat grayish. Others were yellow-brown throughout: larger ones were often roundish with undulate edge. Young colonies were perfectly round, finely granular, brownish discs, with sharply defined edge.

Strain LI.—Macroscopically: like *Ps. alba*. Microscopically: round, entire, yellow-brown, finely granular, concentrically banded or zoned. The edge was comparatively thin.

Ps. longa, and *Ps. mesenterica.*—Macroscopically: like *Ps. alba*. Microscopically: round, brownish discs, somewhat zoned: internally granular becoming nucleated at the center: the periphery was thinner, coarsely granular, and zoned with granular edge. A thin bordering zone showed coiled or curled surface markings. The colonies of the two strains at first were alike, but in 4 days slow liquefaction began in those of *Ps. mesenterica*.

10. *Agar colonies.—Ps. alba.* Growth rapid. Colonies were at first round to irregular, smooth, edge thin and entire.

Microscopically; edge thin, undulate, finely granular (motile organism); grumose towards center. Deep colonies yellow brown and grumose or coarsely granular. Later, macroscopically, the colonies were undulate spreading; microscopically, brown and grumose, the edge ragged, consisting of chains of organisms extending into the medium. Spreading colonies were numerous. (See Plates XV and XVI).

Ps. fluorescens.—Colonies at first punctiform becoming round, irregular and ameboid, characteristically surrounded by a hazy, ill defined, outer zone. Spiny, filamentous processes often fringed the colonies. The internal structure was at first finely granular, becoming coarsely granular to grumose at the center with a finely granular outer zone. Curled, interwoven filaments were frequently seen in the interior. Medium strongly fluorescent.

Ps. longa.—Macroscopically; punctiform, round, becoming irregular or ameboid, spreading, raised and smooth; edge at first entire, later more or less undulate. Microscopically; thin and finely granular, the edge entire to undulate. The internal structure varied from finely to coarsely granular to grumose, floccose or curled.

Ps. mesenterica.—The colonies of this strain could not be distinguished from those of *Ps. longa* except that they were frequently thinner and more widely spreading.

Ps. tenuis.—Punctiform becoming round or irregular. Microscopically; finely to coarsely granular with edge entire to broken and irregular, brownish and usually surrounded by a thin transparent granular zone.

Ps. putrida.—Punctiform to round and irregular, edge entire to broken. Colonies raised, convex, smooth to slightly convoluted. Microscopically; thin, finely granular to grumose, and showing concentric zones with radiate to irregular surface markings; the edge often becoming slightly undulate.

Strain CXII.—Colonies were round to ameboid and frequently thin and spreading. Surface smooth, sometimes concentrically ringed; usually raised, frequently becoming effuse to

hyaline. The edge varied from entire to undulate, frequently thin and spreading. Internal structure, finely to coarsely granular to grumose, often filamentous or floccose; commonly nucleated. Medium strongly fluorescent.

Strain CXL.—Punctiform to round and irregular, typically becoming ameboid spreading, frequently nucleated, somewhat raised to convex, becoming effuse or hyalin. The edge was entire to undulate or broken. Buried colonies irregular with spiny processes. Medium not fluorescent.

Strain CXL. Round to irregular, raised, smooth, entire to broadly undulate, sometimes broken. Microscopically; finely to coarsely granular, grumose towards the center and commonly showing irregular lines, being more or less floccose or curled.

Strain CXLI.—Round, raised to convex, smooth with the edge entire to curled. Microscopically; finely to coarsely granular, becoming grumose; frequently floccose or curled.

Strain CXLIII.—Macroscopically; round, smooth, raised, with edge thin, entire to undulate. Microscopically; finely to coarsely granular or grumose, filamentous and curled to floccose. Medium strongly fluorescent.

Strain XXXVI.—Macroscopically; round, smooth, raised, entire to thin and undulate, frequently becoming effuse. Microscopically; finely to coarsely granular, grumose, filamentous and curled to floccose; sometimes the interwoven chains of cells suggested an anthrax colony. Colonies were often surrounded by a thin transparent zone with an undulate edge.

Strain LI.—Macroscopically; round, irregular to ameboid, smooth, raised with edge entire to undulate. Microscopically; nucleated, finely to coarsely granular, becoming grumose towards the center.

11. *Cohn's nutrient solution* (27:197).—This medium was prepared as follows:

Distilled water	1000.	cc.
Di-potassium phosphate	5.	gr.
Magnesium sulphate	5.	"
Neutral ammonium tartrate	10.	"
Potassium chlorid	0.5	"

It was heated to dissolve the salts, filtered and autoclaved. Growth upon this medium occurred only with *Ps. alba*, *Ps. longa*, and strain LI (also strain LIV identical with strain LI, c. f. page 550). After 20 days, transfers of those failing to develop were made to broth and good typical growth resulted. The development of the three strains upon this medium was accompanied by more or less blue-green to yellow-green fluorescence. Multiple twinned crystals of magnesium ammonium phosphate formed at the surface, which upon attaining sufficient size (about 1 cm. in length), sank to the bottom where they were imbedded in a viscid sediment.

The medium was varied by substituting mono-potassium phosphate for di-potassium phosphate without significant effect upon the development. The character of the growth was the same upon half strength Cohn's solution.

Cultures developed a moderate clouding with white flaky particles in suspension and a white, membranous pellicle bearing crystals on the surface. More or less blue-green fluorescence appeared in the upper portions of the medium. Strain LI (LIV) and *Ps. longa* developed somewhat more slowly than *Ps. alba* and often showed a scum rather than a membranous pellicle.

Typically twinned crystals were constantly associated with development. Similar crystals were artificially formed by exposing sterile tubes of Cohn's solution in a sealed jar with very dilute ammonia. Liter flasks of Cohn's solution were inoculated with strains LI and LIV and a sufficient quantity of the crystals obtained for chemical analysis. The results showed that they were magnesium ammonium phosphate due to the ammonia produced by the bacteria.

12. *Uschinsky solution* (27:197).—The media were prepared as follows:

Distilled water	1000.	cc.
Glycerin (Merck's Blue Label)	40.	gr.
Sodium chlorid	7.	"
Calcium chlorid	1.	"
Magnesium sulphate	0.4	"
Di-potassium phosphate	2.4	"
Ammonium lactate	7.	"
Sodium asparaginate	4.	"

This was filtered and divided into two portions, one of which was used full strength and the other diluted with an equal volume of water. They were autoclaved, and inoculated at the same time from the same stock cultures.

The results attained are summarized in the following table:

TABLE 63. USCHINSKY SOLUTION

Strain	*Normal solution*				*Diluted solution*			
	Growth	Time	Fluorescence	Time	Growth	Time	Fluorescence	Time
Ps. alba,	+	2 d.	+	2 d.	+	1 d.	+	1 d.
Ps. fluorescens,	+	2 d.	+	2 d.	+	2 d.	+	2 d.
Ps. longa,	+	2 d.	±	2 d.	+	1 d.	—	10 d.
Ps. mesenterica,	+	2 d.	+	2 d.	+	1 d.	+	1 d.
Ps. tenuis,	+	2 d.	±	2 d.	+	2 d.	+	2 d.
Ps. putrida,	—	10 d.	—		—	10 d.	—	
CXII,	+	2 d.	+	2 d.	+	1 d.	+	1 d.
CXV,	—	10 d.	—		—	10 d.	—	
CXL,	+	2 d.	—	10 d.	+	1 d	+	1 d.
CXLV,	+	2 d.	+	2 d.	+	1 d.	+	1 d.
CXLVIII,	+	2 d.	+	2 d.	+	1 d.	+	1 d.
XXXVI,	+	2 d.	—		+	1 d.	+	1 d.
LI,	+	10 d.	+	10 d.	+	1 d.	+	2 d.

Modified Uschinsky solution was also prepared as follows:

Distilled water	1000. cc.
Glycerin (Merck's Blue Label)	30. gr.
Sodium chlorid	6. "
Calcium chlorid	0.1 "
Magnesium sulphate (tested purity)	0.4 "
Di-potassium phosphate	2. "
Ammonium lactate	6. "
Sodium asparaginate	4. "

This was filtered and divided into two portions, one of which was used full strength, while the other was diluted with an equal volume of water. Yet another solution was prepared leaving out the ammonium lactate. This was likewise divided into two portions, one being used at full concentration, the other at half concentration. The four media were inoculated at the same time from the same cultures.

The results are tabulated as follows:

TABLE 64. MODIFIED USCHINSKY SOLUTION

Strain	Uschinsky			Half strength Uschinsky		
	Growth	Time	Fluorescence	Growth	Time	Fluorescence
Ps. alba,	+	20 hrs.	+	+	20 hrs.	+
Ps. fluorescens,	+	2 d.	+	+	20 hrs.	+
Ps. longa,	+	20 hrs.	+	+	20 hrs.	—
Ps. mesenterica,	+	2 d.	+	+	20 hrs.	+
Ps. tenuis,	+	20 hrs.	+	+	20 hrs.	+
Ps. putrida,	—	16 d.	—	—	14 d.	—
CXII,	+	20 hrs.	+	+	20 hrs.	+
CXV,	—	16 d.	—	—	14 d.	—
CXL,	+	20 hrs.	+	+	20 hrs.	+
CXLV,	+	20 hrs.	+	+	20 hrs.	+
CXLVIII,	+	20 hrs.	+	+	20 hrs.	+
XXXVI,	+	2 d.	+	+	20 hrs.	—
LI,	+	2 d.	+	+	20 hrs.	+
	Uschinsky, no lactate			Half strength, no lactate		
Ps. alba,	+	20 hrs.	+	+	20 hrs.	+
Ps. fluorescens,	+	20 hrs.	+	+	20 hrs.	+
Ps. longa,	+	20 hrs.	—	+	20 hrs.	+
Ps. mesenterica,	+	20 hrs.	+	+	20 hrs.	+
Ps. tenuis,	+	20 hrs.	+	+	20 hrs.	—
Ps. putrida,	—	14 d.	—	—	14 d.	—
CXII,	+	20 hrs.	+	+	20 hrs.	+
CXV,	—	[1]14 d.	—	+	[1]14 d.	—
CXL,	+	20 hrs.	+	+	14 d.	+
CXLV,	+	20 hrs.	+	+	14 d.	+
CXLVIII,	+	20 hrs.	+	+	14 d.	+
XXXVI,	+	20 hrs.	—	+	14 d.	+
LI,	+	20 hrs.	+	+	14 d.	+

[1] Trace 14 days.

The development of *Ps. fluorescens*, *Ps. mesenterica*, XXXVI, and LI was more rapid but less persistent on the diluted media than on the full concentrations. All strains except *Ps. longa*, *Ps. putrida*, and CXV developed well with surface membrane and fluorescence. *Ps. longa* grew well but without fluorescence; no growth occurred with *Ps. putrida* or CXV. Transfers from tubes of these latter strains to nutrient broth developed typically, showing that the lack of growth on the Uschinsky solutions was not due to a failure to inoculate.

13. *Nitrogen.*—A nitrogen free medium was made as follows (27:198):

Water	500. cc.
Cane sugar	2.5 gr.
Mono-potassium phosphate	1. "
Magnesium sulphate	0.5 "
Sodium chlorid	0.2 "

This was heated until dissolved, and divided into two equal portions. To one was added 2 grams of ammonium tartrate, to the other 2 grams of Bausch and Lomb's peptone.

The results upon these media are tabulated as follows:

TABLE 65. NITROGEN REQUIREMENTS

	Tartrate solution		Peptone solution	
Strain	Growth	Time	Growth	Time
Ps. alba,	—		+	1 d.
Ps. fluorescens,	+	1 d.	+	1 d.
Ps. longa,	—		+	1 d.
Ps. mesenterica,	+	1 d.	+	1 d.
Ps. tennis,	+	1 d.	+	1 d.
Ps. putrida,[1]	+	1 d.	+	3 d.
CXII,	+	3 d.	+	1 d.
CXV,	+	3 d.	+	1 d.
CXL,	+	3 d.	+	1 d.
CXLV,	+	1 d.	+	1 d.
CXLVIII,	+	3 d.	+	1 d.
XXXVI,	+	1 d.	+	1 d.
LI,	+	1 d.	+	1 d.

[1] *Ps. putrida* showed the best growth of any at three days, with beautiful green fluorescence.

In order further to test the nitrogen requirements, the following medium was made, considerable care being taken to make it nitrogen free.

Twice distilled water	3000. cc.
Sucrose	15. gr.
Mono-potassium phosphate (tested purity, *no nitrogen*)	6. "
Magnesium sulphate (tested purity, *no nitrogen*)	0.3 "
Sodium chlorid (tested purity)	1.5 "

After being thoroughly mixed this solution was divided into ten equal portions. One of these was reserved for a control, to another was added 2% of peptone, while to each of the other eight portions was added respectively 0.8% of the following

chemicals: asparagin, sodium asparaginate, urea, potassium nitrate, calcium nitrate, ammonium lactate, ammonium phosphate, ammonium tartrate. The ten media were inoculated at the same time from the same cultures.

TABLE 66. NITROGEN REQUIREMENTS

Strain	Asparagin		Sodium asparaginate		Urea		Peptone		Potassium nitrate	
	G.	F.	G.	F.	G.	F.	G.	F.	G.	F.
Ps. alba,	+	+	+				+	—		
Ps. fluorescens,	+	+	+	+			+	+		
Ps. longa,	+	+	+		—	—	+			
Ps. mesenterica,	+	+	+		+		+	+		
Ps. tenuis	+	+	+				+			
Ps. putrida,							+			
CXII,	+	+	+	+			+	+	+	+
CXV,					—	—	+	—	—	
CXL,	+	+	+		—	—	+	+		
CXLV,	+	+	+		—	—	+	+		
CXLVIII,	+	+	+				+	+		
XXXVI,	+	—	+				+	+	—	
LI,	+	+	+		—		+	+		

Strain	Calcium nitrate		Ammonium lactate		Ammonium phosphate		Ammonium tartrate	
	G.	F.	G.	F.	G.	F.	G.	F.
Ps. alba,			+	+			+	+
Ps. fluorescens,			+	+				
Ps. longa,	—		+			—	+	
Ps. mesenterica,			+	+	+	+	+	
Ps. tenuis,		—	+		+	+	+	
Ps. putrida,		—						
CXII,			+	+				
CXV,								
CXL,			+	+				
CXLV,		—	+	+			—	
CXLVIII,			+	+	—	—		
XXXVI,			+		+	+	+	
LI,		—	+	+			+	—

NOTE.—G., growth; F., fluorescence; +, observed; —, not observed.

Observations upon these media were continued for over a month. Peptone was the only source of nitrogen used by all strains. Except with strain CXV, good growth was apparent in 24 hours; the latter showed good growth in 3 days. All strains, except *Ps. putrida* and CXV, were able to utilize asparagin and

sodium asparaginate. Upon ammonium lactate the results were practically the same; however, with *Ps. longa* and LI, growth was somewhat delayed. *Ps. alba, Ps. longa, Ps. mesenterica, Ps. tenuis,* XXXVI, and LI, used ammonium tartrate as a source of nitrogen. Upon the ammonium phosphate medium *Ps. mesenterica, Ps. tenuis,* and XXXVI, developed. With potassium nitrate, strain CXII showed a delayed growth. None of the strains developed on the calcium nitrate medium. *Ps. mesenterica* was the only strain which developed upon the medium containing urea.

III. Physical and Biochemical Features.

For the purpose of determining acid production triplicate titrations of cultures and controls were made after 1, 2, 4, 10, and 20 day incubation periods. Five cc. of the medium were pipetted into 45 cc. of distilled water in Erlenmeyer flasks, boiled two minutes and titrated hot with N/20 sodium hydroxid against phenolphthalein.

1. *Sugar free medium.*—Control titrations were made upon a sugar free medium prepared as follows: 1% of Bausch & Lomb's peptone was added to distilled water and steamed in a double cooker about 50 minutes, tubed 10 cc. per tube, and autoclaved. A small amount of acid was produced on this sugar free medium by strains *Ps. putrida,* CXII, CXV, CXLVIII. Previous to boiling the acid was masked by ammonia. The figures in the table indicate the percent of normal acid or alkali produced.

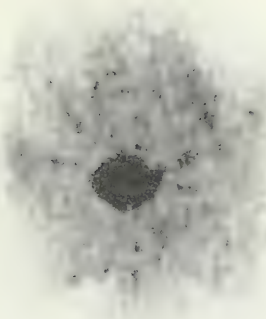

PLATE XV.—Types of agar colonies. Figure 1. *Ps. alba* (two day old colony showing anthrax-like strands). Figure 2. *Ps. fluorescens*, strain CXLVIII (two day old colony). (See pages 568-570.)

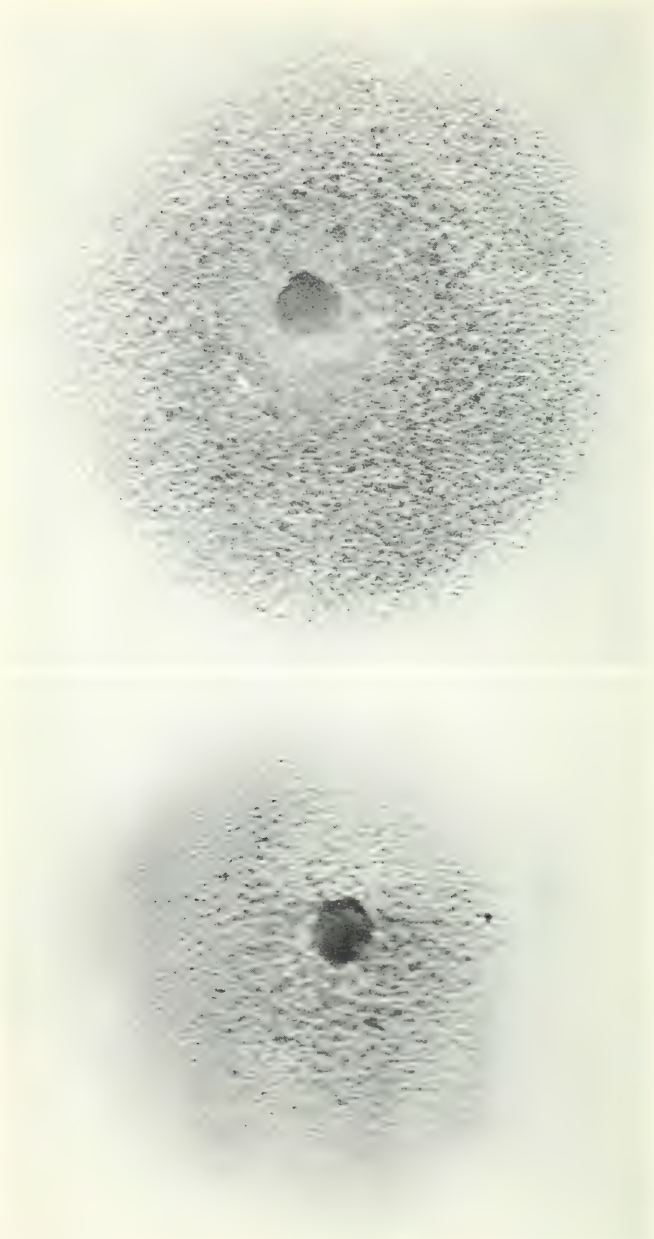

PLATE XVI.—Types of agar colonies. *Ps. fluorescens var. non-liquifaciens*, strain CXL; two day old colonies, showing grumose structure. (See pages 568-570.) × 40.

TABLE 67. PERCENT NORMAL ACID OR ALKALI PRODUCED IN SUGAR FREE MEDIUM

Strain	1 day		2 days		4 days		10 days		20 days	
	Acid	Alk.	Acid	Alk.	Acid	Alk.	Acid	Alk.	Acid	Alk.
Ps, alba,		0.09		0.21		0.14		0.36		0.24
Ps. fluorescens,		0.05		0.13		0.15		0.15	0.02	
Ps. longa,		0.09		0.15		0.10		0.29		0.27
Ps. mesenterica,		0.16		0.14		0.20		0.31		0.28
Ps. tenuis,		0.12		0.10		0.18		0.25		0.24
Ps. putrida,		0.04		0.12		0.06	0.09		0.53	
CXII,		0.09	0.00	0.00	0.05			0.14	0.22	
CXV,		0.01		0.06		0.04	0.02		0.34	
CXL,		0.06		0.12		0.06		0.29		0.23
CXLV,		0.05		0.10		0.18		0.34		0.19
CXLVIII,		0.04		0.06	0.03		0.16		0.47	
XXXVI,		0.06		0.07		0.18		0.24		0.28
LI,		0.02		0.07		0.10		0.25		0.20

2. *Sugar media.*—To 1% peptone solutions identical with the control, 2% of dextrose, lactose, sucrose, and glycerin respectively were added. The several media were treated like the control except that they were sterilized by the intermittent method. Analytical results obtained with these four media are shown in tabular form. In general, previous to boiling, the acid produced was masked by the ammonia formed.

TABLE 68. PERCENT NORMAL ACID OR ALKALI PRODUCED ON DEXTROSE PEPTONE

Strain	1 day		2 days		4 days		10 days		20 days	
	Acid	Alk.	Acid	Alk.	Acid	Alk.	Acid	Alk.	Acid	Alk.
Ps. alba,	0.02		0.06		0.08			0.06		0.11
Ps. fluor.,	0.03		0.15		0.14		0.08		0.09	
Ps. longa,		0.02		0.03	0.03			0.03		0.04
Ps. mes.,	0.12		0.14		0.26		0.29		0.36	
Ps. tenuis,	0.12		0.12		0.14		0.31		0.67	
Ps. putrida,	0.00	0.00		0.03	0.00	0.00		0.03	0.00	0.00
CXII,	0.21		0.30		0.35		0.38		0.49	
CXV,	0.00	0.00		0.09	0.00	0.00	0.02		0.00	0.00
CXL,	0.00	0.00	0.01		0.13		0.25		0.28	
CXLV,	0.05		0.08		0.24		0.07		0.16	
CXLVIII,	0.05		0.16		0.30		0.44		0.52	
XXXVI,	0.11		0.07		0.10		0.00	0.00	0.00	0.00
LI,		0.02		0.02	0.05		0.00	0.00	0.00	0.00

PERCENT NORMAL ACID OR ALKALI PRODUCED ON LACTOSE PEPTONE

	1 day		2 days		3 days		10 days		20 days	
Strain	Acid	Alk.	Acid	Alk.	Acid	Alk.	Acid	Alk.	Acid	Alk.
Ps. alba,		0.10		0.21		0.23		0.23		0.15
Ps. fluor.,		0.16		0.24		0.20		0.04	0.19	
Ps. longa,	0.00	0.00		0.05		0.14		0.20		0.17
Ps. mes.,		0.10		0.13		0.16		0.21		0.22
Ps. tenuis,		0.06		0.14		0.13		0.15		0.15
Ps. putrida,		0.07		0.04		0.08	0.03		0.46	
CXII,		0.08		0.06	0.11		0.25		0.41	
CXV,		0.02		0.05		0.06	0.05		0.36	
CXL,		0.08		0.16		0.12		0.12	0.09	
CXLV,		0.11		0.15		0.15		0.21		0.15
CXLVIII,	0.02			0.02	0.15		0.41		0.93	
XXXVI,		0.10		0.13		0.09		0.09	0.04	
LI,	0.00	0.00		0.07		0.11		0.11	0.01	

PERCENT NORMAL ACID OR ALKALI PRODUCED ON SUCROSE PEPTONE

	1 day		2 days		4 days		10 days		20 days	
Strain	Acid	Alk.	Acid	Alk.	Acid	Alk.	Acid	Alk.	Acid	Alk.
Ps. alba,	0.03			0.19		0.12		0.15		0.25
Ps. fluor.,		0.05		0.05		0.16	0.09		0.08	
Ps. longa,	0.02		0.00	0.00		0.09		0.10		0.17
Ps. mes.,		0.03		0.08		0.12		0.14		0.17
Ps. tenuis,	0.00	0.00	0.05			0.09		0.07		0.15
Ps putrida,	0.02		0.00	0.00		0.11	0.35			0.11
CXII,	0.06		0.20		0.24		0.65		0.56	
CXV,	0.06		0.05		0.00	0.00	0.08		0.10	
CXL,	0.01		0.03			0.09		0.11		0.17
CXLV,	0.00	0.00		0.03		0.12		0.14		0.16
CXLVIII,	0.00	0.00	0.00	0.00	0.15		0.58		0.60	
XXXVI,	0.03			0.01		0.15	0.02			0.01
LI,	0.04			0.06		0.14		0.09		0.14

PERCENT NORMAL ACID OR ALKALI PRODUCED ON GLYCERIN PEPTONE

	1 day		2 days		4 days		10 days		20 days	
Strain	Acid	Alk.	Acid	Alk.	Acid	Alk.	Acid	Alk.	Acid	Alk.
Ps. alba,		0.12		0.19		0.21		0.24		0.19
Ps. fluorescens,		0.07		0.13		0.06	0.08		0.36	
Ps. longa,		0.05		0.13		0.14		0.28		0.22
Ps. mesenterica,		0.07		0.15		0.17		0.24		0.05
Ps. tenuis,		0.01		0.12		0.16		0.13		0.11
Ps. putrida,		0.02	0.04			0.02	0.22		0.71	
CXII,		0.10		0.03		0.02	0.12		0.26	
CXV,	0.00	0.00	0.03		0.03		0.07		0.20	
CXL,		0.04		0.17		0.19		0.18		0.16
CXLV,		0.11		0.16		0.12		0.26		0.16
CXLVIII,		0.01		0.03	0.13		0.69		0.87	
XXXVI,		0.04		0.14		0.02	0.13		0.29	
LI,		0.05		0.14		0.08		0.22		0.14

3. *Action on nitrates in nitrate broth.*—Medium:

Water	1000. cc.
Peptone (Witte's)	1. gr.
Potassium nitrate, c. p.	2. "

The cultures were tested for nitrites by the iodo-starch reaction as follows (27:63). A starch paste was made by boiling a 4% aqueous mixture of potato starch; to the 10 cc. of culture 1 cc. of the starch paste and 1 cc. of freshly prepared potasium iodid water (0.4%) were added, followed by three drops of sulphuric acid (2 parts c. p. sulphuric acid to 1 part water). One series of cultures and controls was tested upon the second, fifth, and ninth days.

A diphenylamine reagent was prepared as follows (32:349): 5 grams of diphenylamine were dissolved in 100 cc. of pure concentrated sulphuric acid and added to 20 cc. of water. In case of negative nitrite tests with the iodo-starch reaction, a few drops of diphenylamine reagent were poured down the side of the tube in such a manner as to form 2 layers. A blue contact zone indicated the presence of nitrates. When a positive nitrite reaction was observed the nitrate test was performed with another culture.

Second day.—A positive nitrite reaction (immediate blackening) was given by *Ps. mesenterica*, XXXVI, and LI. Nitrates were present in all by the diphenylamine reaction. Ammonia was indicated in all except CXV by Nessler's reagent.

Fifth day.—An immediate reaction for nitrites was given by *Ps. mesenterica*, XXXVI, and LI. Nitrates and ammonia were indicated in all.

Ninth day.—Immediate positive reaction for nitrites occurred in cultures of *Ps. mesenterica*, XXXVI, and LI and *Ps. fluorescens* showed traces. Nitrates were indicated in all. A positive reaction for ammonia was obtained from all cultures, especially strong in *Ps. putrida* and CXLVIII.

A nitrate medium in which tap water was substituted for distilled water was employed in fermentation tubes. The tests were conducted as before, about 10 cc. of the medium being poured into clean test tubes, for this purpose.

Sixth day.—Immediate nitrite reaction occurred in the following: *Ps. mesenterica,* XXXVI, LI and a trace in *Ps. fluorescens,* while nitrates were found in all. No gas was observed. A test for nitrites was also made by the naphthlamine-sulphanilic acid reagent. A positive reaction was obtained with *Ps. mesenterica,* XXXVI, LI, and *Ps. fluorescens.* In the latter the color was not as deep as in the others.

Eleventh day.—Only slight clouding in the open arm was observed; no gas was produced. A positive nitrite reaction was obtained with *Ps. fluorescens, Ps. mesenterica,* XXXVI, and LI. Nitrates and ammonia were present in all by the diphenylamine and Nessler's reagents respectively. A strong reaction with Nessler's reagent was given by *Ps. putrida, Ps. fluorescens,* CXII, and CXLVIII.

4. *Indol.*—The tests for indol on nutrient broth and on Dunham's peptone solution were made by adding 10 drops of chemically pure sulphuric acid and 1 cc. of .02% sodium nitrite to 10 cc. of culture. The tubes were allowed to stand 10 minutes and then heated. Strains *Ps. alba* and CXL gave a trace of a reaction in 10 minutes without heating. The results on nutrient broth are given in table 69 and those on Dunham's peptone solution in table 70. A positive result is indicated by +, a negative by —, and a trace by ±.

TABLE 69. INDOL PRODUCTION IN NUTRIENT BROTH.

Strain	10 d.	13 d.	18 d.	20 d.	22 d.	24 d.	26 d.	30 d.	30 d.	30 d.	37 d.	40 d.	40 d.	40 d.	60 d.
Ps. alba,	—	+	+	+	+	+	—	+	±	+	—	±	±	+	+
Ps. fluor.,	—	—	—	—	—	—	—	—	—	—	—	±	—	—	—
Ps. longa,	—	—	—	±	—	—	±	±	±	±	—	—	—	—	+
Ps. mes.,	—	—	—	—	—	—	—	—		—	—	—	—	—	±
Ps. tenuis,	—	—	—	—	—	—	—			—	—	—	—	—	±
Ps. putrida,	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
CXII,	—	—	—	—	—	—	—	—	—	—	—	—	—	—	±
CXV,	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
CXL,	+	+	+	+	+	+	+	+	+	+	±	+	+	+	+
CXLV,	—	—	—	—	—	—	—	—	—	—	—	—	—	—	±
CXLVIII,	—	—	—	—	—	—	—	—	±	—	—	—	—	—	—
XXXVI,	—	—	—	—	—	—	—	—	±	—	—	±	—	—	—
LI,	—	—	—	—	—	±	±	±	±	±	—	±		—	

TABLE 70. INDOL PRODUCTION IN DUNHAM'S PEPTONE

Strain.	2 days	6 days	11 days	26 days
Ps. alba,	—	—	±	+
Ps. fluorescens,	—	—	—	±
Ps. longa,	—	—	—	±
Ps. mesenterica,	—	—	—	—
Ps. tenuis,	—	—	—	—
Ps. putrida,	—	—	—	—
CXII,	—	—	—	—
CXV,	—	—	±	±
CXL,	—	—	+	+
CXLV,	—	—	—	—
CXLVIII,	—	—	—	±
XXXVI,	—	—	—	—
LI,	—	—	—	—

5. *Toleration of acids and alkalies.*—Three liters of nutrient broth were prepared in the usual way and divided into 13 portions. These separate lots were adjusted, by titrating hot against phenolphthalein with N/20 sodium hydroxid and N/20 hydrochloric acid, to the following reactions: —37.5; —30.0; —23.8; —13.5; —11.0; —7.3; 0.0; +8.8; +14.6; +25.0; +34.0 Fuller's scale.

Since alkaline media rapidly change in reaction owing to carbon dioxid absorption, control tubes of the several media were subjected to the same conditions as the cultures and their reactions determined after 1 and 3 days. Table 71 indicates the results secured upon these alkali and acid media after 24 hours' incubation. The original reactions of the several media and, also, the reactions after 24 hours are given at the top of the table. Table 72 indicates the reactions of the media and the growth of the organisms after a 3 days' incubation.

TABLE 71. GROWTH ON ACID AND ALKALI MEDIA AFTER 24 HOURS

	Reaction of media previous to tubing (Fuller's Scale)					
	0.0	7.3	—11.0	—13.5	—23.8	—37.0
	Reaction after 24 hours. (Fuller's scale)					
Strain	+0.13	—2.0	— 4.9	— 7.4	—14.2	—17.9
Ps. alba.	+	+				
Ps. fluorescens.	+					
Ps. longa.	+					
Ps. mesenterica.	+					
Ps. tenuis.	±	+				
Ps. putrida.	+	+	+			
CXII,	+	+				
CXV,						
CXL,	+	+				
CXLV,	+	+				
CXLVIII,						
XXXVI,	+	±				
LI,						

	Reaction of media previous to tubing (Fuller's Scale)				
	—37.5	+8.8	+14.6	+25.0	+34.0
	Reaction after 24 hours. (Fuller's scale)				
Strain	—22.1	+8.7	+12.5	+22.0	+33.7
Ps. alba.		+	+	±	
Ps. fluorescens.		+	+		
Ps. longa.		+	+		
Ps. mesenterica.		+	+		
Ps. tenuis.		+	+		
Ps. putrida.		+	+		
CXII,		+	+		
CXV,					
CXL,		+	+		
CXLV,		+	+		
CXLVIII,		+	+		
XXXVI,		+	+		
LI,		+	+		

+, growth; —, no growth; ±, doubtful growth.

TABLE 72. GROWTH ON ACID AND ALKALI MEDIA AFTER 3 DAYS

Strain	Reaction after 3 days. (Fuller's scale) +3.0	+0.4	−2.2	−3.3	−10.0	−14.6
Ps. alba.	+	+				
Ps. fluorescens.	+	+	+	—	—	—
Ps. longa.	+	+	—	—	—	—
Ps. mesenterica.	+	+	—			
Ps. tenuis.	+	+				
Ps. putrida.	+	+	+	+	—	—
CXII.	+	+	±			
CXV.						
CXL.	+	+				
CXLV.	+	+				
CXLVIII.	+					
XXXVI.	+	+		—	—	—
LI.	+					—

Strain	Reaction after 3 days. (Fuller's scale) −20.0	+10.9	+13.5	+24.1	+34.6
Ps. alba.		+	+	+	
Ps. fluorescens.	—	+	+	+	
Ps. longa.		+	+		
Ps. mesenterica.		+	+	+	—
Ps. tenuis.		+	+		
Ps. putrida.		+	+		—
CXII.		+	+	+	
CXV.			+		
CXL.		+	+		
CXLV.		+	+	+	
CXLVIII.		+	+	+	
XXXVI.		+	+	+	—
LI.	—	+	+		—

+, growth; —, no growth; ±, doubtful growth.

6. *Optimum reaction for growth in bouillon.*—In connection with the experiments on the toleration of sodium hydroxid and hydrochloric acid, observations were made after a day's incubation as to the optimum reaction for growth. The best growth with all strains was found to occur in a very slightly acid medium. The figures given in table 73 represent the original reaction of the media in which growth occurred in 24 hours (c. f. page 582 for actual reactions). The reactions at which the best growth occurred are shown in black face.

TABLE 73. OPTIMUM REACTION

Strain			Reactions		
Ps. alba,			0.0	+8.8	+14.6
Ps. fluorescens,		—7.3	**0.0**	−8.8	+14.6
Ps. longa,			0.0	+8.8	+14.6
Ps. mesenterica,			0.0	+8.8	+14.6
Ps. tenuis,			0.0	+8.8	+14.6
Ps. putrida,	—11.0	—7.3	**0.0**	+8.8	+14.6
CXII,			**0.0**	+8.8	+14.6
CXV,[1]					
CXL,			0.0	+8.8	
CXLV,		—7.3[2]	**0.0**	+8.8	+14.6
CXLVIII,			0.0	+8.8	+14.6
XXXVI,			0.0	+8.8	+14.6
LI,			0.0	+8.8	+14.6

[1] No growth at 24 hours.
[2] Trace of growth.

7. *Vitality on culture media.*—Long vitality on culture media was characteristic. Five month agar cultures completely dried down, developed promptly with fluorescence when transferred to nutrient broth.

8. *Temperature relations; thermal death point.*—Thin walled tubes of uniform thickness with an inside diameter of 15 to 17 mm. were chosen for the determinations of the thermal death point. Seven cc. of bouillon, +10 Fuller's scale, were employed in each case. The inoculations were made from young broth cultures, care being taken not to wet the tube above the surface of the medium. In the preliminary determinations the tubes were deeply submerged in the thermal bath for 10 minutes at the following temperatures: 44.5, 47.7, 54.5, 58.4, 61.3° C. After exposure the tubes were incubated at 25° C. In this way the thermal death point for all strains was roughly fixed between 47.7 and 54.4° C. None grew after exposure at 54.5° C.

In subsequent trials the temperature of the bath was varied so as to determine the death point more closely. A very large

number of tests were made in an effort to fix the exact death point accurately, but the experiments indicate clearly that the death point of these organisms under the conditions employed, is not constant within at least 0.5° C. The final conclusions are recorded in the table below:

TABLE 74. THERMAL DEATH POINT

Strain		°C.		°C.
Ps. alba,	between	53.5	and	53.9
Ps. fluorescens,	"	53.1	"	53.5
Ps. putrida,	"	53.5	"	53.9
Ps. longa,	"	51.1	"	51.5
CXL,	"	51.5	"	51.8
CXLVIII,	"	51.5	"	52.9
CXLV,	"	50.2	"	50.5
CXII,	"	50.2	"	51.5
CXV,	"	50.2	"	50.5
XXXVI,	"	50.2	"	50.5
Ps. mesenterica,	"	49.3	"	50.2
LI,	"	49.1	"	49.3
Ps. tenuis,	"	48.3	"	49.1

Optimum temperature.—The best growth with all strains occurred at approximately 25° C.

Maximum temperature.—At 36.7 to 35° C. only *Ps. fluorescens* and *Ps. putrida* developed in 24 hours. At 33.4 to 30.8° in the same length of time *Ps. alba, Ps. fluorescens, Pr. longa, Ps. mesenterica, Ps. putrida,* CXII, and CXL, showed growth. At 31.2 to 28.5° *Ps. alba, Ps. fluorescens, Ps. longa, Ps. mesenterica, Ps. tenuis, Ps. putrida,* CXII, CXL, and LI developed. The experiments indicated that all strains could grow at temperatures up to 33° C. Only *Ps. fluorescens, Ps. putrida,* and CXV developed at 36° C. in 7 days. In no case was the growth at these higher temperatures as good as it was below 30° C.

9. *Drying.*—Small cover slips were placed on bits of paper in petri dishes and sterilized in the oven. Each was then inoculated with one loop of young broth culture, the age of which varied in the different experiments from 1 to 3 days. They were allowed to remain in the dark at room temperature. At intervals, cover slips were removed and dropped into tubes of

sterile broth. A characteristic growth after incubation was regarded as evidence of continued vitality. It is to be noted that in general the organisms retained their vitality longer when taken from the older cultures than when take from day old cultures.

TABLE 75. RESISTANCE TO DESICCATION

Strain	Days dried								
	1	2	2	2	3	3	3	4	4
Ps. alba.			+	—	+	+	—	+	+
Ps. fluorescens.	+		+	+			+		
Ps. longa.	+		+				—		
Ps. mesenterica.	+			+			—		
Ps. tenuis.									
Ps. putrida.	+		+	+	+	+	+	+	+
CXII,	+		+	+	+	+	+	+	+
CXV,					+	—			
CXL,	+	+	+				—		
CXLV,	+	+	+	—			+	+	+
CXLVIII,	+		+	+	+	+	+	+	+
XXXVI,	+	+	+	+	+	+	+	+	—
LI,	+	+		+			—		

Strain	Days dried								
	6	6	6	10	14	18	20	30	47
Ps. alba.						—		—	
Ps. fluorescens.						—		—	
Ps. longa.						—		—	—
Ps. mesenterica.						—			
Ps. tenuis.						—			
Ps. putrida.		+	+	+		+		+	
CXII,	+	+	+	+		+			
CXV,					—				
CXL,	+				—	—	—		—
CXLV,					—	+	—	—	—
CXLVIII,		+	+	+		+		+	—
XXXVI,		+	+		—	+	—	—	—
LI,	+				+	—	—	—	

+, growth; —, no growth; ±, doubtful growth.

10. *Insolation.*—Agar plates, one-half covered were exposed on snow to bright sunlight for 10 minutes at 11 a. m. Feb. 29. All were sensitive, the percent killed varying from 23 to 100%. The number of colonies on the unexposed half.

the number on the exposed half, and the percent killed are indicated below:

TABLE 76. INFLUENCE OF INSOLATION

Strain	Number of colonies on unexposed half	Number of colonies on exposed half	Percent killed
Ps. alba,	225	70	69
Ps. fluorescens,	340	100	70
Ps. longa,	68	21	69
Ps. mesenterica[1]	24	2	92
Ps. tenuis,	280	18	93
Ps. putrida,	numerous	0	100
CXII,	18	0	100
CXV,	280	56	80
CXL,	numerous	0	100
CXLV,	186	142	23
CXLVIII,[1]	8	2	75
XXXVI,[1]	countless	42	95
LI,	numerous	0	100

[1] Percent could only be estimated on account of spreaders.

Ten minutes exposure of strains CXII, CXV, CXL, CXLV, CXLVIII, XXXVI, and LI, March 8, at 11 a. m. gave results indicated in table 77.

TABLE 77. INFLUENCE OF INSOLATION

Strain	Plate No.	Number of colonies on unexposed half	Number of colonies on exposed half	Percent killed
CXII,	1	350	0	100
	2	413	0	100
CXV,	1	38	0	100
	2	6	0	100
CXL,	1	1494	0	100
	2	2289	0	100
CXLV,	1	381	0	100
	2	540	0	100
CXLVIII,	1	318	127	60
	2			
5,	1	540	226	58
	2	763	85	89
XXXIII,	1	countless	0	100
	2	"	0	100
XXXVI,	1	381	0	100
	2			
LI,	1	158	0	100
	2	222	1	99.5

11. *Acids produced.*—Hydrogen sulphid. Tubes of nutrient broth were prepared and inoculated in the ordinary way and in each, including controls, was suspended a strip of filter paper thoroughly moistened with lead acetate solution. Blackening of the paper after a suitable incubation of the cultures indicated the presence of hydrogen sulphid. The following strains gave this reaction constantly, CXV, CXL, *Ps. tenuis.* With strain CXV it was in evidence in 2 days; with *Ps. tenuis* and CXL, in 5 to 6 days. A slight reaction was observed in tubes of LI and a doubtful trace occasionally in *Ps. putrida,* CXII, and CXLV.

12. *Alkalics produced.*—Ammonia was indicated by Nessler's reagent in nitrate broth cultures of all strains (c. f. page 579).

13. *Crystals.*—Crystals of magnesium ammonium phosphate were formed in Cohn's solution by *Ps. alba, Ps. longa* and strain LI.

14. *Diastasic action on potato starch.*—Broth cultures of the 13 strains were tested for diastasic action upon potato starch after incubation periods of varying duration. The results were negative.

15. *Anaerobiosis.*—Pyrogallic acid oxygen absorption method: Freshly prepared agar slants were placed in a liter Novy jar containing 15 grams of pyrogallic acid. Just before sealing about 200 cc. of a 1% sodium hydroxid solution was added. After 10 days incubation in the sealed jars a slight growth with fluorescence was apparent in strains *Ps. alba, Ps. fluorescens, Ps. longa, Ps. mesentcrica, Ps. tenuis,* CXII, CXL, CXLV, CXLVIII, XXXVI, and LI. No growth resulted with *Ps. putrida,* or CXV; however, normal growth occurred in tubes of the latter two strains soon after they were removed from the jar.

Carbon dioxid method.—Carbon dioxid was produced from washed and boiled marble and hydrochloric acid in a Kipp generator and purified by passing successively through solutions of

sodium carbonate, potassium permanganate, pyrogallic acid in 1% sodium hydroxid, and distilled water and allowed to pass for 2 hours through a Novy jar containing freshly prepared agar slants. The jar was sealed and held at room temperature. No strain showed growth after 5 days incubation, but in all cases good growth occurred in from 1 to 2 days after the seal was broken. A second series incubated for 10 days gave duplicate results. In addition to the plain agar slants, lactose and dextrose agar slants were used with similar results. The reaction of the incubated agar medium was roughly determined by triturating 5 cc. of the solid medium in 45 cc. of distilled water and titrating. Sufficient carbon dioxid had been absorbed to give the cold medium a reaction of +20 Fuller's scale.

In order to be sure that the failure to grow in the carbon dioxid was not due to the acidity of the medium, augmented by absorbed carbon dioxid, the following anaerobic methods were employed.

Hydrogen method.—A series of plain agar slants and a series of 1% dextrose litmus agar slants were placed in a Novy jar and the air displaced by hydrogen. The jar was exhausted in order to hasten the displacement. The litmus agar remained neutral, and titrations of the plain agar controls without boiling demonstrated that the reaction had not been changed. No growth occurred in 10 days. All developed well when removed from the jar.

Roux method.—Sterilized Roux tubes were aseptically filled with young broth cultures of the 13 strains. This test was tried several times and in no case did growth appear except in defective tubes or in those containing air bubbles. After incubation of 10 days the tubes were broken and the contents gathered in sterile test tubes where normal growth resulted.

These tests show that the various strains are strictly aerobic. They further indicate that lactose and dextrose are not fermented in the absence of oxygen. (Consult papers of Andrewes and Hordu (1) and Glenn (10) in bibliography).

GROUP NUMBER OF THE 13 STRAINS

The group number of the 13 strains was easily computed with the exception of the digits representing the action of the bacteria upon carbohydrates and glycerin. On account of the formation in the control, i. e., peptone solution, of decomposition products having an acid reaction, there is some doubt in certain cases whether the acid in the sugar and glycerin peptone media resulted from oxidation of the introduced compounds, or from proteid decomposition. In each case the amount of acid produced in the carbohydrate media was compared with the amount, if any, produced in the control, and where the difference was less than 0.2% the digit representing no acid production was recorded. However a comparison of the tables will show that no sharp line of differentiation exists between acid production and no acid production.

The group numbers determined were as follows:

Ps. alba,	212.2332133	Non-liquefiers
Ps. longa,	212.3332133	
Ps. putrida,	212.3332132	
Ps. mesenterica,	211.2333133	Tardy liquefiers
Ps. LI,	211.2233133	
Ps. tenuis,	211.2322133	
Ps. CXL,	211.2222133	
Ps. fluorescens,	211.2223132	Rapid liquefiers
Ps. CXII,	211.2222133	
Ps. CXLV,	211.2332133	
Ps. CXLVIII,	211.2222132	
Ps. XXXVI,	211.2223132	
B. CXV,	211.3332?33	Doubtfully fluorescent

SPECIES

As already noted bacteriological literature contains descriptions of over 50 species of bacteria which are capable of producing green fluorescence upon the common media. However, it is probable that many so-called species are identical with, or varieties of, previously named species. The following references from literature are cited as illustrations of the trend of opinion.

Niederkorn (23) concluded that there are only two constant forms, *B. fluorescens liquefaciens* Flugge and *B. pyocyaneus* Gessard, among the following:

Bacillus pyocyaneus.		a	Gessard.
"	"	b	Ernst.
"	"	c	Freudenreich.
"	"	*pericardit.*	Harold-Ernst.
"	"		vom bakt. Institut. Travel.
"	"		strumit. Lanz, Bern.
"	*fluorescens*	*liquefaciens*	Flügge.
"	"	*albus*	Adametz.
"	"	*aureus*	Zimmermann.
"	"	*longus*	"
"	"	*tenuis*	"
"	"	*mesentericus*	Tataroff.
"	"	*putridus*	Flügge.
"	"	*capsulatus*	Pottien.
"	"	*liquefaciens*	(vom Verf. aus Wasser isoliert).

Ruzicka (24) shows that *B. pyocyaneus* and *B. fluorescens liquefaciens* vary so widely within the species and so overlap in their cultural characters that no sharp line of differentiation between them can be drawn.

Griffon (11) states that *B. caulivorous, B. brassicacvorous* and *B. aeruginosus* are not to be considered as distinct species, but only as forms of *B. fluorescens* which, under a favorable environment, easily changes from a saprophytic to a parasitic existence. He holds that *B. aeruginosus* is to be considered synonymous with *B. fluorescens putridus.*

According to various authors: *B. fluorescens non-liquefaciens is* synonymous with *B. fluorescens putridus* and representative of the group comprising *Bacillus fluorescens tenuis, Bacillus fluorescens aureus* and *Bacillus fluorescens crassus; Bacillus viscosus* Frankland is synonymous with *Bacillus fluorescens liquefaciens,* and *Bacillus fluorescens fuscus* corresponds to *Bacillus oogenes fluorescens.*

In view of the above citations it is evident that the fluorescent organisms are closely related, probably being varieties of one polymorphic species. Before stating the conclusions which have been drawn concerning the number of species represented in the seven strains of sap bacteria and the six known strains

which were studied, attention is directed to a few clauses abstracted from Flügge's description of his *Bacillus fluorescens liquefaciens* (6:II: 292). "The degree of liquefaction varied very considerably. There were varieties which in 2 days and others which first in 2 weeks liquefied the gelatin; the form of the colonies varied, also, from spherical to spreading with border entire to toothed. The optimum temperature was 20° to 25° C. Many varieties do not grow at 37° C, others grow well and form on agar a more or less thick layer. The oxygen requirements are equally variable; through them are explained the different forms of liquefaction in stab cultures. If one wishes to conceive all minor variations as constant characters, one must make dozens of varieties."

According to Flügge (l. c.) *Bact. butyri fluorescens* Lafar, *B. fluorescens nivalis* Schmelck, *Bacillus viscosus* Frankland and *B. fluorescens minutissimus* Unna may be regarded as synonymous with *B. fluorescens liquefaciens.* In the description of *B. fluorescens non-liquefaciens*, he says, "A sharp line between the *liquefaciens* and *non-liquefaciens* variety of *B. fluorescens* does not exist since there are forms which in the first day of plate growth appear to belong to the latter group, which later sink gradually in the gelatin."

It is believed that the morphological, cultural, physical and biochemical features of the 13 strains of green fluorescent bacteria warrant the assumption that there are but two species represented: *Pseudomonas fluorescens* and an unknown species, strain CXV, which, as has been previously noted (c. f. page 546), never evidenced more than doubtful fluorescence.

Ps. fluorescens is represented by 12 strains which are divisible into two varieties, *liquefaciens* and *non-liquefaciens.* The following strains belong to the *liquefaciens* variety: *fluorescens mesenterica*, CXII, CXLV, CXLVIII, XXXVI and LI. To the *non-liquefaciens* variety belong, *alba, longa, putrida* and, provisionally, *tenuis* and strain CXL, which showed a very tardy

liquefaction of gelatin but in other respects closely resemble the other three strains.

New species.—Strain CXV: This strain appears to be different from any of the others studied. A feeble trace of fluorescence was thought to have been present at times in agar cultures. Repeated staining for flagella has shown that the latter are peritrichiate. Another peculiarity about this organism is the parallel grouping of single or double cells common in capsule and flagella preparations, c. f. Plate X, figures 3 and 4. As previously noted several cells commonly occurred side by side in the same capsule with several flagella arising about the periphery of the latter. A careful consideration of the character of this bacillus seems to justify the conclusion that it is a distinct species. The name *Bacillus parallelus* is proposed for this organism. For purposes of reference the differential characters of this new species are summarized as follows:

BRIEF DESCRIPTION OF BACILLUS PARALLELUS

(NEW SPECIES)

I. MORPHOLOGICAL CHARACTERS

1. *Form.*—A short motile bacillus with rounded ends occurring singly, typically in twos; long chains common upon solid media.

2. *Size.*—Individual cells had the following dimensions. On hanging blocks; length 1 to 2.6 microns, diameter .8 to 1.0 microns. Hanging drops; length 1.0 to 4.0 microns, diameter .5 to .9 micron, stained preparations; length .8 to 2. microns, diameter .5 to .8 microns.

3. *Grouping.*—Short and long chains; delicate membranous pellicle.

4. *Endospores.*—None observed.

5. The flagella were peritrichiate: tufts were common at each end of short chains, but lateral flagella were also present on what appeared to be individual cells. Easily demonstrated by Löwit's or Loeffler's method.

6. *Capsules.*—Capsules easily demonstrated upon preparations from 10 day broth cultures by Welch's method or Richard Muir's contrast stain. Frequently 2 and as many as 5 cells occurred side by side within the same capsule with flagella arising about the periphery of the envelope.

7. *Zoogloea.*—Small zoogloea masses were common in liquid cultures.

8. *Staining reactions.*—Gram's stain: Deeply stained even after 15 minutes in absolute alcohol; with amyl alcohol, Gram's stain was irregularly retained after 5 minutes' exposure. Aqueous fuchsin: deeply stained. Aqueous gentian violet: well stained. Carbol fuchsin (Ziehl): well stained.

II. CULTURAL FEATURES

1. *Agar stroke.*—Not characteristic; a narrow filiform, white slimy to viscous band. Doubtful fluorescence of the medium.

2. *Potato.*—A narrow, slimy, filiform band, becoming brownish; sometimes the growth became dull and more or less rhizoid in character; the medium was more or less grayed.

3. *Agar stab.*—Best growth at top, colony at first white, becoming brownish; development somewhat restricted; the line of puncture was at first beaded to filiform, becoming somewhat villous. The medium showed a very slight fluorescence and in old cultures became amber brown.

4. *Gelatin stab.*—Growth best at the top; line of puncture filiform to beaded; liquefaction at first crateriform, later stratiform, beginning in 24 hours and proceeding rapidly at first; complete in 62 days.

5. *Nutrient broth.*—Rather slow growth; moderate uniform clouding in from 2 to 5 days; transient; white membranous coherent precipitate; old cultures were brownish and stringy.

6. *Milk.*—Digestion apparent in about 4 days; a firm coagulum appeared in about 18 days accompanied by acid production; digestion was completed in from 30 to 104 days; the medium never appeared green but was more or less straw colored throughout.

7. *Litmus milk.*—Alkaline reaction in 4 days, succeeded by slight acid production when digestion began; the litmus was completely reduced in 2 or 3 weeks' time.

8. *Gelatin colony.*—Rapid growth; first punctiform to round and entire, rapidly sinking in the saucer-like depressions of the liquefying gelatin.

9. *Agar colonies.*—Punctiform and round to irregular and ameboid; the colonies effuse or raised and convex; the edge thin entire to undulate and broken; finely to coarsely granular to grumose; commonly nucleated. Colonies dense, dark brown, fringed with spiny processes occurred less frequently.

10. *Cohn's solution.*—No growth.

11. *Uschinsky solution.*—No growth.

12. *Nitrogen requirements.*—Nitrogen obtained from peptone and ammonium tartrate.

III. PHYSICAL AND BIOCHEMICAL FEATURES

1. *Action upon carbohydrates in peptone solution.*—Neither acid nor gas were formed in 1% peptone solutions containing 2% of either dextrose, lactose, sucrose or glycerin.

2. *Ammonia production.*—Moderate ammonia production; 18.6 cc. N/10 hydrochloric acid equivalent produced in 100 cc. of broth in 10 days.

3. *Nitrates in nitrate broth.*—Not reduced. Nitrates and ammonia present; ammonia probably from proteid decomposition.

4. *Indol production.*—Doubtful in 11 day Dunham's peptone cultures after boiling; absent in nutrient broth.

5. *Toleration of acids and alkalies.*—Slight.

6. *Optimum reaction for growth.* +15 Fuller's scale.

7. *Vitality on culture media.*—Long.

8. *Temperature relations.*—Thermal death point; between 50.2 and 50.5° C. Optimum temperature approximately 25° C.: maximum, 37° C; minimum, 5-7° C.

9. *Resistance to desiccation.*—Continued vitality after 3 days drying on cover slips; 4 days fatal.

10. *Insolation.*—Sensitive; 80 to 100% killed after 10 minutes' exposure.

11. *Acids produced.*—Abundant hydrogen sulphid production.

12. *Alkalies.*—Moderate ammonia production.

13. *Relation to oxygen.*—Strict aerobe.

14. *Group number.*—Bacillus 211.3332?33.

BRIEF CHARACTERIZATION OF THE 12 STRAINS OF *Ps. fluorescens*

For the convenience of any one who may have occasion to review these results the brief characterization items of the six strains of fluorescent bacteria isolated from maple sap together with those of strains *alba, fluorescens, longa, mesenterica, tenuis,* and *putrida* are summarized in tabular form on pages 598 and 599.

CONCLUSIONS

Among the 42 strains of green fluorescent bacteria selected from several hundred strains which had been isolated from maple sap, there were 32 strains of the *liquefaciens* and 9 strains of the *non-liquefaciens* varieties of *Ps. fluorescens*. The studies included one strain which was never more than doubtfully fluorescent which, for this and other reasons, should not properly be regarded as a member of the fluorescent group. The 9 strains of the *non-liquefaciens* variety showed a delayed liquefaction of gelatin in from 50 days to 5 months when cultivated in a moist chamber at 20° C. Peptonization of milk by these strains was also long delayed commencing in from 30 days to 3 months.

Critical comparative studies of 7 representative strains of green fluorescent sap bacteria and 6 so-called species *Ps. alba*, *Ps. fluorescens*, *Ps. longa*, *Ps. mesenterica*, *Ps. tenuis*, and *Ps. putrida* show that no sharp line of differentiation can be drawn between these forms. Of the known "species," *Ps. alba*, *Ps. longa*, and *Ps. putrida* fail to liquefy gelatin in 6 months time, while in the case of *Ps. mesenterica* and *Ps. tenuis* a delayed liquefaction occurs. In the latter named strain liquefaction first appears 4 months after inoculation.

It is believed that the fluorescent sap bacteria as well as the so called species, *Ps. alba*, *Ps. longa*, *Ps. mesenterica*, *Ps. tenuis*, and *Ps. putrida* should properly be recognized as strains of the *liquefaciens* and *non-liquefaciens* varieties of *Ps. fluorescens*.

TABLE 78. BRIEF CHARACTERIZATION

MORPHOLOGY		Diameter over 1 micron
		Chains
		Endospores
		Capsules
		Pseudozoogloea
		Motile
		Gram's stain
CULTURAL FEATURES	Broth	Cloudy
		Pellicle
		Sediment
	Agar Gel. plate	Shining
		Round
	Gel. Stab	Surface-growth
		Needle-growth
	Potato	Moderate
		Discolored
		Grows, at 37° C.
		Grows, in Cohn's solution
		Grows, in Uschinsky solution
BIOCHEMICAL FEATURES	Liquefaction	Gelatin [1]
		Casein
		Agar
	Milk	Coagulated [2]
		Casein peptonized [3]
		Indol
		Hydrogen sulphid
		Ammonia
		Nitrates reduced
		Fluorescent

[1] Under observation 6 months.
[2] Coagulum more or less jelly-like in consistency.
[3] Under observation 4 months.

OF THE 12 STRAINS OF *Ps. fluorescens*.

Ps. alba	*Ps. fluorescens*	*Ps. longa*	*Ps. mesentérica*	*Ps. tenuis*	*Ps. putrida*	Strain CXII	Strain CXL	Strain CXLV	Strain CXLVIII	Strain XXXVI.	Strain LI
+	+	+	+	+	+	+	+	+	+	+	+
						—	—	+	?	?	—
+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+
+	+				+						
+		+									+
+	+	+	+	+		+	+	+	+	+	+
	+		+	+		+	+	+	+	+	+
	+					+			+	+	
+	+	+	+	+	+	+	+	+	+	+	+
+							+				
+	+	+	+	+	+	+	+	+	+	+	+
	+		+							+	+
+	+	+	+	+	+	+	+	+	+	+	+

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